Vol. 39 No. 4

BULLETIN

OF THE

TORREY BOTANICAL CLUB

APRIL 1912

Methods of culture and the morphology of the archicarp in certain species of the Ascobolaceae

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(WITH PLATES 10–15)

INTRODUCTION

The method of origin of the ascocarp in the Ascobolaceae has been an interesting subject of investigation since the days when De Bary and his contemporaries discovered the presence of specially differentiated hyphae, ascogonia, etc., in young fruit bodies of certain Ascomycetes. If the reports of those who have studied the origin of the ascocarp can be accepted, we have in this one family of the Ascobolaceae a wide range of variation. In *Ascodesmis* a cell of the mycelium gives rise to a group of spirally coiled ascogonia (Claussen, 1905). A similar cluster of antheridia arises from the same mycelium and each antheridium becomes twisted about an ascogonium. Fertilization is brought about through the fusion of the one-celled trichogyne with the antheridium. In *Thelebolus*, according to Ramlow (1906), the mycelium gives rise to an ascogonium which is at first one-celled but which later becomes several-celled by the formation of cross walls. The single ascus of the ascocarp arises from the penultimate cell of the ascogonium. Between these two widely different methods of ascocarp formation others have been described which seem to furnish evidence of a gradual loss of sexuality and the assumption of an apogamous or parthenogenetic development.

I have undertaken a general investigation of several species of

[The BULLETIN for March 1912 (39: 85–138. *pl.* 5–9) was issued 18 Ap 1912.]

the group, growing them as far as possible in pure cultures, thus making possible a more extended and comparative study of the method of the origin of the ascocarp in the endeavor to furnish further data for an understanding of the relationships existing within and between the various groups of the Ascomycetes.

One obstacle in the way of a more thorough study of the life history of the species of this group has been the great difficulty encountered in all attempts to germinate the ascospores and thus obtain pure cultures in artificial media. I have, therefore, given this phase of the question special attention in my work.

REVIEW OF THE LITERATURE

The literature relating to the question of sexuality in the Ascomycetes has been many times thoroughly reviewed in recent years. I need only refer to the careful and critical papers of Wager (1899), Dangeard (1903, 1907), Vuillemin (1907), Claussen (1907), and Guilliermond (1908, 1910). Data in the literature bearing on the germination of the spores and the comparative morphology of the different forms of ascogonia occurring in the different genera of the Ascobolaceae have not been so thoroughly summarized, and I shall attempt to bring together the available material in this line.

Coemans (1862) claimed that the spores of all species of *Ascobolus* germinate readily on moist slides and produce a mycelium not unlike that of other fungi. He considered remarkable the great facility with which this mycelium produced such quantities of *Penicillium*- or *Torula*-like conidia.

Woronin (1866), who was unable to germinate the spores of *Ascobolus*, questioned the correctness of the account given by Coemans. He described the initial organ of *Lasiobolus pulcherrimus* as a vermiform body consisting of about ten or twelve large cells arising perpendicularly from a vegetative hypha and curving slightly to one side. The cells are rather dark colored, and in addition to the granular contents each cell has one or more vacuoles. As the vermiform body (scolecite) reaches maturity three or four cells at the outer end are surrounded by several hyphae, which send out short sickle-shaped branches cut off from the parent hypha by septa, so that each branch consists of an oblong cell

borne on a short stalk. These branches are closely applied to the cells of the scolecite in such a way as to lead Woronin to believe that fertilization occurs at this time.

He further found chlamydospores or resting spores on the mycelium connected with the ascocarp but no conidia or oidia. These thick-walled brownish spores are borne on curved stalks. They germinate after a resting period of several weeks.

Boudier (1869) believed that the spores of the species of *Ascobolus* growing on dung germinate only after passing through the digestive canal of animals, and that the spores of species growing on earth germinate at ordinary temperatures without special treatment. His figure of a germinated spore of *Ascobolus carbonarius* (*A. viridis* Boud.) shows the characteristic mycelium produced by this species. He asserts that in spite of all his work on this group he has never found their mycelia producing anything in the nature of conidia or chlamydospores.

Janczewski (1871) is very positive in his refutation of Coemans' statement (1862) regarding the germination of *Ascobolus* spores without special treatment. He fed the ascocarps of *A. furfuraceus* to an animal, recovering the germinated spores from the faeces. He found that most of the epispore is removed by the digestive processes, and that the spores thus treated germinate, sending out one or more germ tubes at a short distance from each end of the spore. He also investigated the method of the formation of the ascocarp. The material for this purpose was obtained directly from the natural substratum. The ascogonium consists of eight or ten large cells formed as a "worm-like" branch of a vegetative hypha. A slender filament arising from near the base of the ascogonium branches several times and coils closely about the end cells. These filamentous hyphae are the "pollinodia." After the ascogonium is well developed, the third cell from the outer end of the archicarp is seen to be very much enlarged and gives rise to ascogenous hyphae from its upper surface.

Van Tieghem (1876) germinated the spores of *Ascodesmis nigricans* in dung decoctions and beerwort. Following their development in drop cultures he observed the formation of a T-shaped body which branched several times by a false dichotomy. This rosette is the initial organ of the ascocarp. Occasionally a second

branch arising near the first and similar to it, takes part in the formation of what he considered a compound or double ascocarp. Claussen (1905) has shown that this second branch is the stalk of a compound antheridium.

Borzi (1878) found a "scolecite" present in the young ascocarps of *Lasiobolus equinus* (*Ascophanus pilosus*), *Ascobolus immersus*, and *Rhyparobius* sp. The mature "scolecite" of *Lasiobolus equinus* consists of eight or ten cells which are differentiated into three distinct regions. The first eight cells form the stalk, which is usually bent a little to one side. The central portion consists of one large spherical cell borne on this stalk. The third region is merely a small projecting cell topping the ascogenous cell. The "pollinodium" is a filamentous hypha which grows up along the stalk of the "scolecite" and branches two or three times as it reaches the terminal portion, the branches coiling tightly about the end cell. Borzi considered the "pollinodia" to be concerned with a process of fertilization and at this time believed sexual reproduction was common among the Ascomycetes. He further finds that *L. equinus* produces two kinds of asexual spores. The first kind is borne on slender, erect, septate stalks. They are thick-walled brownish resting spores which he calls chlamydospores. The second kind appears to be borne on rather thick brushlike branches of erect hyphae. The spores are pinched off from the tips of these branches as small hyaline conidium-like "spermatia." In *Ascobolus immersus* the eight-celled stalk bears a large spherical cell, which is also capped by a small projecting cell. He finds two or three large cells in the young ascocarps of *Rhyparobius* sp. These cells are only the remains of the "scolecite." He figures the asci as arising directly out of the larger cell.

Zukal (1889) cultivated *A. immersus* by a series of transfers on sterilized dung, making the final transfers to a dung decoction where he was able to observe the various stages of growth under the microscope. He found two kinds of asexual spores with heavy brown walls. Branches composed of five or six cells arise from the older mycelial hyphae; the end cell rounds up and becomes surrounded with a thick brown membrane. If the end cell becomes divided by a longitudinal wall in addition to two or three transverse walls, a spore similar to those produced in *Stemphylium*

is formed. He describes the ascocarp as originating in a knot of ordinary mycelial hyphae without the appearance of sex organs or an ascogonium. He denies the existence of the large ascogonium which Borzi found in this species. Zukal (l. c.) germinated the spores of *Rhyparobius pachyascus* in dung decoction and followed the development of the ascocarp in drop cultures. He strongly denies the presence of sex organs, but his figure of the initial organs might well be taken to represent oogonia and antheridia. One cell of the mycelium becomes slightly larger than the neighboring cells and sends out a large oval bud which is soon cut off from the parent cell by a septum. Two or three branches arise from the base of this large oval cell, and curving inward, become closely applied to its apex. He states that the mycelium of *Rhyparobius Cookei* gives rise to what he calls "gemmae," by intercalary swellings. He finds that spores of *Ascophanus saccharinus* germinate readily in Liebig's meat extract. The ascocarp originates from a knot of hyphae which he calls a sclerotium. After about six days portions of the mycelial hyphae swell up into knots, which increase in size by a process of budding, forming a compact mass about 40 μ in diameter. These so-called sclerotia may develop directly into ascocarps or they may continue to increase in size up to 80 μ, become reddish in color, and remain in this condition several weeks before developing an ascocarp. A second kind of sclerotium is more parenchymatous than the first and contains a large central cell. Other sclerotia about 200 μ in diameter are covered with hairlike appendages. These sclerotia are said to develop ascocarps if kept in moist chambers about six weeks.

Brefeld (1901) grew *Thelebolus stercoreus* in dung decoctions. He figures a small coiled ascogonium of one or two turns. *Rhyparobius albidus* was the only other species of this family which he was able to cultivate from ascospores. By using portions of the mycelium of *Ascobolus denudatus* he obtained good cultures of the species. The mycelium produced great quantities of oidia. After a time, when the production of oidia had ceased, an abundant crop of apothecia developed. He was able to trace the connection of the apothecia with the mycelium giving rise to the oidia. The oidia were sown on sterilized horse dung, and apothecia were obtained. He does not describe the manner in which the fruit body originates.

Harper (1896) describes the ascogonium of *Ascobolus furfuraceus* as it appears from the time it is completely inclosed with investing hyphae. In this stage the ascogonium consists of a coil of several large cells. Large pores are already formed in the transverse walls, thus bringing the contents of the cells of the row into direct connection. The ascogenous hyphae develop from the entire surface of the ascogenous cell.

Miss Ternetz (1900) finds that ascospores of *Ascophanus carneus* germinate readily in dung decoctions or in dung decoction agar. Cultures kept in a dark room do not develop fruit. The ascogonium arises as a branch of the mycelium. The stalk consists of two to five cells, easily distinguished from the larger and more granular cells of the ascogonium. The coil makes one or two turns, including in this region six or eight cells, and ends in a septate filament which varies considerably in length. It usually contains two or three cells but it may continue to grow out and give rise to another ascogonium. She found no evidence of the existence of an antheridium.

Massee and Salmon (1901, 1902) conducted a series of experiments on spore germination in species of *Ascobolus*. They found that the spores of *Ascobolus albidus* and *A. perplexans* would not germinate at 16° C. in dung decoction but did germinate at 27° C. *A. albidus* grown on dung in a closed tin box, shot the spores on the cover of the box, where they germinated and are said to have produced ascocarps. Spores taken from the ascocarps on the box cover were grown in a nutrient medium, and ascocarps were developed. They found that spores from these ascocarps would not germinate after passing through the alimentary canal of a guinea pig but did germinate at ordinary temperatures in tap water. They drew the very doubtful conclusion from this, that all dung-growing species of *Ascobolus* originally grew on earth where the spores would germinate easily at low temperatures. After one generation on the cover of the box and a second in an artificial medium made of plum jam and gelatin, *Ascobolus albidus* had reverted to its original terrestrial habit.

Molliard (1903) believes that the presence of certain bacteria favors the production of ascocarps by *Ascobolus*. He germinated the spores of *A. furfuraceus* in some way which he does not describe,

and obtained pure cultures on sterilized cow dung and on slices of carrot. A dense growth of white flocculose mycelium was produced which gave rise to a great number of what he termed "arthrospores." In rare cases, after several weeks a few ascocarps appeared in some of the cultures. He discovered that the same bacteria that were present on the dung from which he obtained the ascospores for inoculation were also present in these particular cultures. Pure cultures of the *Ascobolus* mycelium and of this bacterium were then made, and he had only to introduce the bacteria into this *Ascobolus* culture in order to obtain an abundant supply of ascocarps. Otherwise he claims the *Ascobolus* remained sterile indefinitely.

Barker (1904) describes the existence of sex organs in *Rhyparobius* sp. but gives no figures. The ascogonium is a spirally coiled branch containing five or six nuclei. An antheridium containing several nuclei arises from the cell next to the one producing the ascogonium. The antheridium grows up and its tip becomes attached to the end of the ascogonium. Septa are now formed in both sex organs, cutting off cells which are uninucleated. The penultimate cell of the ascogonium is, however, binucleated.

Claussen (1905) grew cultures of *Ascobolus furfuraceus*, in which he found chains of oidia produced on the mycelium. He traced a direct connection between the mycelium from a germinated ascospore and the oidia. He sowed these oidia and continued the cultures in this manner for a hundred generations without finding any ascocarps. He has also studied in especial detail the life history of *Ascodesmis nigricans*. This fungus is easily cultivated on artificial media. He finds that the rosette of ascogonia originates as a result of the dichotomous branching of an outgrowth of a vegetative hypha. A short bud is put forth from a cell of the mycelium and immediately becomes T-shaped by division. Branching continues until several pairs of spirally coiled ascogonia are formed. Each ascogonium is now cut off from its stalk by a septum and a one-celled trichogyne is cut off at the apex. The antheridia are produced on a branch which may arise from the next cell to that from which the ascogonial branch originates, or it may come from cells of other hyphae in the vicinity. The antheridial branch divides dichotomously and grows in among the

ascogonia, one antheridium coiling spirally about each ascogonium. Fusion takes place between the trichogyne and the antheridium.

According to Ramlow (1906) the mature ascogonium of *Thelebolus stercoreus* is a more or less spirally coiled body composed of five or six cells. Each cell is uninucleated except the penultimate cell, which contains two nuclei. The ascus is formed directly from the penultimate cell of the ascogonium. He was unable to find any organ corresponding to an antheridium. Occasionally two or three asci are found in one apothecium. This results from the inclusion of as many separate ascogonia within one apothecium.

Overton (1906) finds that *Thecotheus Pelletieri* has a compound fruit body containing several ascogonia. Each ascogonium is composed of several multinucleated cells and resembles in form the ascogonium of *Ascobolus*. He is unable to find any opening between the adjacent cells of the ascogonium. The ascogenous hyphae may arise from any or all the cells of the ascogonium.

Dangeard (1907) has described observations on many of the species of Ascobolaceae and related Discomycetes. In what he regards as *Ascophanus ochraceus* he finds that the ascocarp originates from a rosette of eight or ten ascogonia. These ascogonia resemble those of *Pyronema* although they are somewhat smaller. The ascogonium is prolonged into one or two elongated cells and these continue as a slender filament bending back and over the ascogonium. He claims that this outgrowth does not correspond to a trichogyne as no antheridium is present. The mature ascogonium of *Ascobolus furfuraceus* consists of about ten cells. It arises as a lateral branch from the mycelium and as growth proceeds is cut up into multinucleated cells. As it develops it becomes more or less coiled, forming an irregular spiral of one or two turns. He does not find the large pores in the transverse walls reported by Harper (1896), on the contrary he finds that the pores are just such as occur in the septa of the vegetative hyphae, too minute to allow of a bodily transfer of nuclei and cytoplasm. The asci are formed from the binucleated cells of the ascogenous hyphae. The method of forming the ascogonium in a species that he thinks may be *Ascobolus glaber*, differs widely from that of *A. furfuraceus*. The ascogonium has a stalk of twenty or thirty cells of much less diameter than the cells of

the parent hypha. The stalk grows along on the medium and forms the spiral coil at its extremity. Dangeard has not been able to follow what takes place in the tangled coil, except to find that its cells are multinucleated and are connected by minute pores. A species which he accidentally found in his cultures of *Pyronema*, did not mature. It showed an exceedingly large ascogonium with the three regions, which I shall discuss later, easily recognizable.

Miss Fraser (1907) developed a method for germinating the spores of *Lachnea stercorea*. She believes that in nature the spores of this species probably pass through the body of the cow. An attempt was made to imitate normal digestion by placing the spores successively in saliva, artificially prepared gastric juice, pancreatic juice, and in a dung decoction. The spores were left in each medium several hours and the temperature maintained at 38° C., the temperature of the cow. Germination occurred after about two days. Further experiments showed that the two factors most essential for germination were heat and the alkalinity of the medium. Five successful experiments on germination by this method are recorded. Her figure of the germinated spore shows a germ tube issuing from each end. She was unable to obtain ascocarps, as all growth ceased soon after the germ tubes were formed. The mature ascogonium of *Lachnea stercorea* consists of a large oval cell at the end of a three- or four-celled stalk. A trichogyne grows out from one side of the ascogonium, and transverse septa are formed in the trichogyne, cutting off several cells. The tip of the trichogyne bends downward and becomes attached to a more or less irregular and indefinite antheridium, the origin of which could not be determined. According to Miss Fraser the septa of the trichogyne are permanent structures, and functional sexuality does not exist.

Miss Fraser's method of spore germination was used by Miss Welsford (1907) in her studies of *Ascobolus furfuraceus* and by Cutting (1909) working with *Ascophanus carneus*. They describe the germination of a few spores but were unable to obtain artificial cultures. Miss Welsford found thick-walled chlamydospores developed on a mycelium supposed to be that of *Ascobolus furfuraceus*, and Cutting observed the "gemmae" and "dense chains

TABLE I

Summary of results obtained by various investigators who have reported germinating the spores of certain species of the Ascobolaceae. Those marked (?) have given no figure of the germinated spores nor a description of the manner in which the spores germinated.

Date	Author	Species	Method	Results
1862	?Coemans	*Ascobolus* sp.	In water	Mycelium producing many conidia
1869	Boudier	*Ascobolus carbonarius* (*A. viridis* Boud.)		No further growth noted
1871	Janczewski	*A. furfuraceus*	Animal digestion	No further growth noted
1876	Van Tieghem	*Ascodesmis nigricans*	In drop culture dung decoction	Produced ascocarps
1889	?Zukal	*Rhyparobius pachyascus*	Dung decoction	Produced ascocarps
1889	?Zukal	*R. Cookei*		Produced "gemmae" and ascocarps
1889	?Zukal	*Ascophanus saccharinus*	Liebig's Beef Extract	Produced 3 kinds of "sclerotia" and ascocarps
1891	Brefeld	*Thelebolus stercoreus*	In dung decoction	Produced ascogonia
1891	Brefeld	*Rhyparobius albidus*	In dung decoction	Produced ascocarps
1900	?Ternetz	*Ascophanus carneus*	In dung decoction agar	Produced "gemmae" and ascocarps
1902	Massee & Salmon	*Ascobolus albidus*	In tap water and plum juice	Produced ascocarps
1902	?Massee & Salmon	*Ascobolus perplexans*	In tap water and plum juice	
1903	?Molliard	*Ascobolus furfuraceus*	On sterilized carrot and dung	Produced ascocarps and arthrospores
1906	?Barker	*Rhyparobius* sp.	In dung decoction	Produced ascocarps
1906	Ramlow	*Thelebolus stercoreus*	In dung decoction	Produced ascocarps
1907	?Claussen	*Ascobolus furfuraceus*		Produced oidia. No ascocarps
1907	?Welsford	*Ascobolus furfuraceus*	Dung decoction + Na_2CO_3 at 38° C.	No further growth
1909	?Cutting	*Ascophanus carneus*	Dung decoction + Na_2CO_3 at 38° C.	No further growth

of chlamydospores" noted by Miss Ternetz (1900) in connection with the mycelium of *Ascophanus carneus*.

TABLE I does not include the names of several who have cultivated *Ascodesmis* from the germinated spores: Zukal (1885), Claussen (1905), Dangeard (1907), Bainier (1907). It is evident that the spores of *Rhyparobius* germinate readily in dung decoction and those of *Thelebolus stercoreus* can be germinated with difficulty in the same medium. The only satisfactory evidence that spores of species of *Ascobolus* have been germinated in artificial media is furnished by Boudier (*A. carbonarius*), Janczewski (*A. furfuraceus*), and Massee & Salmon (*A. albidus*). Janczewski's method involved the process of the digestion of the spores by the animal and is not practicable for artificial culture work. Boudier germinated the spores of *A. carbonarius* but reported no further growth. Massee & Salmon report that they obtained fruits of *Ascobolus* artificially in cultures started by the germination of the ascospores. As previously noted, page 143, Brefeld obtained abundant growths of apothecia of *A. denudatus* from mycelium that also produced oidia. Zukal (1889) grew *Ascobolus immersus* by a series of transfers on sterilized dung. Dangeard (1907) reports growing several species of *Ascobolus* in artificial cultures but he does not describe his methods.

Various terms are now in use to designate the initial organs of the ascocarp. It is difficult to choose a term that shall include such simple fertile hyphae as exist in the Erysiphaceae, *Monascus*, and *Pyronema* and at the same time the complicated structures found in *Aspergillus*, in species of the Ascobolaceae, and in the lichens. The word archicarp will be used in this discussion when referring to the branch consisting wholly or in part of the oogonium or its morphological equivalent, the oogonium being that organ which produces the egg that is fertilized.

In *Sphaerotheca* the oogonium is a uninucleated cell (Harper, 1896), in *Pyronema* it is multinucleated (Harper, 1900). In *Ascophanus carneus*, according to Cutting (1909), there is a fusion of vegetative nuclei in each of four or five cells of the archicarp, but such variations, if they occur, do not necessarily affect the morphological equivalence of the organs in question.

The trichogyne is an outgrowth of the oogonium and functions

as an organ to bring together in the oogonium the male and the female nuclei.

The term ascogonium will be used in referring to the oogonium after fertilization. In such cases as *Pyronema* the oogonium after fertilization becomes the ascogonium. Such a use of terms is of course purely morphological and does not prejudge the question as to the existence of apogamy, reduced fertilization, etc.

It is necessary only to mention the antheridium in the lichens, in *Pyronema*, and in *Monascus*, to call to mind the great variation which may occur in this organ, and it would perhaps be premature to attempt to substitute a single term in place of the wide variety of terms that have been proposed.

MATERIAL AND METHODS

The Ascobolaceae form a rather natural group as commonly regarded by systematists. The family as now generally recognized contains about two hundred described species, grouped under ten or twelve genera. The genus *Ascobolus* was established by Persoon (Gmel. Syst. Nat. **2**: 1461. 1791) to include those Discomycetes in which the mature asci protrude from the fruit body.

Several noteworthy monographs dealing with the species of this family have been published in Europe, and all morphological and cytological papers on the group have been based on European material. A few species have been described from America but our knowledge of the group as it exists here is extremely limited. In my preliminary work to obtain suitable material for study I succeeded in growing on the natural substrata in damp chambers thirty-six species and a few additional forms which appear to be varieties.

Cultures were carried on both at the New York Botanical Garden and at Columbia University where most of the artificial culture work was done. I am indebted to Professor C. C. Curtis for his liberality in the provision of apparatus and opportunities to visit other regions for the purpose of collecting these fungi. Professor R. A. Harper's criticisms and timely suggestions deserve an expression of sincere appreciation.

Extensive field studies were carried on and the plants were grown under natural as well as artificial conditions. In the field

studies of such species as *Ascobolus viridis*, *A. pusillus*, and *A. carbonarius*, all of which grow on the ground rather than on the excrement of animals, repeated visits to the same spots for three successive years were made and the condition of the species under various weather conditions was noted. Apothecia in all stages of growth were brought into the laboratory and their diagnostic characteristics were worked out in detail. For laboratory cultures on the natural substratum, earth and dung were brought in and placed in damp chambers of glass lined with filter paper.

Some species are very minute, and the apothecia are frequently so few that their detection is a matter of difficulty. The Zeiss binocular with the horizontal arm is a very useful instrument for this work. With the higher powers, and a damp chamber provided with a flat cover, it is possible to study the development of the plants, their heliotropic reactions, and the discharge of the spores under very normal conditions.

The apothecia seem to develop best when the cultures are carried on in a well lighted room having a temperature of about 25°–27° C. In a dimly lighted or cold room there are usually marked changes in the coloration of the spores and fruit bodies. *Ascophanus carneus* when grown in bright sunlight will have a deep pink apothecium, but in a dimly lighted room the fruit body will be nearly colorless. The spores of *A. Winteri* that were allowed to develop in a closed vasculum were perfectly hyaline. When this species is grown in a cold room or is found in nature during cold rainy weather, the spores are much paler than usual.

In order to determine whether certain species were limited in their occurrence to a particular kind of dung, extensive studies were made of the species growing on dung from the New York Zoological Park. So far as could be learned, most of the species are not thus limited.

The following species have been grown on their natural substrata in the laboratory. Their identity has been determined by careful study and comparison with exsiccati at the New York Botanical Garden:

Ascobolus aerugineus Fries
Ascobolus carbonarius Karst.
Ascobolus sp.
Ascobolus furfuraceus Pers.
Ascobolus glaber Pers.
Ascobolus immersus Pers.
Ascobolus Leveillei Boud.

Ascobolus Leveillei var. *americanus* Cooke & Ellis
Ascobolus pusillus Boud.
Ascobolus viridis Currey var. (?)
Ascobolus viridulus Phil. & Plow.
Ascobolus Winteri Rehm
Ascophanus Aurora (Crouan) Boud.
Ascophanus carneus (Pers.) Boud.
Ascophanus sarcobius Boud.
Ascophanus glaucellus Rehm
Ascophanus granuliformis (Crouan) Boud.
Ascophanus Holmskjoldii Hansen
Ascophanus lacteus (Cooke & Phil.) Phil.
Ascophanus microsporus (B. & Br.) Phil.
Ascophanus minutissimus Boud.
Ascophanus ochraceus (Crouan) Boud.
Ascodesmis nigricans v. Tieghem (*Boudiera Claussenii* P. Henn.)
Boudiera sp.
Cubomia sp.
Thecotheus Pelletieri (Crouan) Boud.
Rhyparobius crustaceus (Fuckel) Rehm
Rhyparobius pachyascus Zukal
Rhyparobius niveus (Fuckel) Rehm
Rhyparobius sexdecimsporus (Crouan) Sacc.
Lasiobolus equinus (Müll.) Karst.
Saccobolus depauperatus (B. & Br.) Rehm
Saccobolus Kerverni (Crouan) Boud.
Saccobolus neglectus Boud.
Saccobolus violaceus Boud.
Thelebolus stercoreus Tode

Of this list fourteen species have been grown more extensively and studied from the morphological standpoint as to the characters of their ascogonia, methods and conditions of spore germination, etc. Further studies are in progress, dealing with nuclear phenomena and methods of reproduction. The discussion of the identification of these species forms a part of another paper, which is now being prepared.

I have found aceto-carmin useful for staining young ascogonia, although it is not permanent, and as a temporary stain it can not always be depended upon to differentiate between the nuclei and the granules of the cells. The nuclei are more easily differentiated in the young hyphae. A drop of the aceto-carmin mixture placed on a piece of agar containing the fungus before it is crushed under the cover glass, will stain the hyphae and ascogonia, if the slide is left for some time in a damp chamber where the stain does not dry out. The ascogonia, especially, swell under the action of the stain but this is no disadvantage when it is desired only to locate them.

Material stained in toto with iron-hematoxylin can be crushed on the slide, perfectly dehydrated, and mounted in balsam without shrinkage. Such mounts are useful for showing germinated spores and the young mycelium. A still better method is to allow the spores to be shot upon a slide where they will stick securely enough to be carried through all the processes of heating, germination, fixation, etc.

For the serial sections, Heidenhain's iron-hematoxylin and Flemming's triple stain were used. If longitudinal sections of hyphae were desired the blocks were cut parallel to the original upper surface of the culture medium. The ascogonia could also be located more easily in sections cut in this plane. The coils of the ascogonia do not appear to be oriented in any particular plane, and a section parallel to the upper surface of the agar is as favorable as any for their study.

The nutrient medium most frequently used for artificial cultures was made by soaking 12–14 g. of agar over night in 500 c.c. of tap water and then adding to it 500 c.c. of filtered decoction of goose dung, obtained by allowing about 100 g. of the dung to remain in a liter of warm water for a few minutes. The mixture was heated in an autoclave at 120° C. for 30–40 minutes or in a steam sterilizer for a longer time. It was then filtered with a hot water filter and further sterilized for 30 minutes at 120° C., or intermittently for three days at 100° C. Another nutrient decoction used with good results when a medium with little color was desired, was made by heating about 2 kg. of common garden soil in an oven at 180° C. for an hour. A liter of a filtrate obtained from this soil while still warm was added to 12 g. of agar.

The decoctions of goose dung were always strongly alkaline. During the sterilizing process the ammonia was largely driven off so that the medium was only very slightly alkaline to litmus. After the ascocarps had ripened on the medium, tests made with litmus gave sometimes an acid and sometimes an alkaline reaction, the one occurring as often as the other. The medium made with an extract of heated soil was very slightly acid. Petri dishes 5–10 cm. in diameter and 1–2 cm. high, with as thin bottoms as could be obtained, were preferred as culture dishes on account of the method of observation employed. The plates were poured so that the medium was about 3 mm. deep and left to harden without disturbing.

SPORE GERMINATION

As a preliminary to obtaining artificial cultures of as large a number of species as possible, experiments were made to determine the most favorable conditions for the germination of the spores.

A quantity of goose dung gathered in August 1910, at the Zoological Park, was brought into the laboratory and placed in a tall battery jar completely lined with filter paper, and the jar was left uncovered to facilitate the partial drying of this freshly gathered material. It was left in this condition four or five days. The odor of ammonia, which had been very strong the first few days, became gradually less noticeable, but the bacterial decomposition appeared to be still going on rapidly. Examination showed, however, that the entire surface of the dung was covered with a dense aggregation of young fruits of *Ascobolus Winteri* Rehm.

ASCOBOLUS WINTERI Rehm.

Agar plates were prepared as described above, the nutrient being a decoction of goose dung. The effect of various temperatures, ranging from the body temperature of the birds downward, was first tested. For this purpose a copper germinating trough was used. This trough contained ten compartments covered with glass plates. It was heated by electricity so that the successive compartments were held at temperatures of 42°, 40°, 38°, 35°, 30°, 25°, 25°, and 24° C. respectively. Petri dishes containing the agar inoculated with spores, were placed in these chambers and examined from time to time for 48 hours with negative results in all cases. The plates were then set aside at this time and examined for two or three days or until they became contaminated with bacteria and other fungi. In no case could any germinating spores be found, though the conditions as to degree of heat and time of exposure must have resembled those in the bird. This experiment was tried several times with minor variations. Sometimes a low percentage of sodium carbonate or various combinations of other salts were added to the decoction used in making up the medium. The spores could not be stimulated to growth under any of these conditions.

Some of the damp chambers in which the fungus was growing stood on a laboratory table exposed to direct sunlight. In these cases spores were occasionally found that had germinated in the film of moisture that always gathers on the underside of slides placed over the dung to catch the spores. An attempt was made

to transfer these germinated spores to the agar plates. When the spores were removed from the slide the germ tubes were either broken off or injured in some manner, but if the slides themselves were placed on the medium the mycelium would continue to grow and finally produce ascocarps. These cultures invariably became contaminated with foreign fungi.

Small pieces of dung or filter paper upon which young ascocarps were growing, were next placed in the agar medium. Although many attempts were made to obtain cultures in this manner, not the slightest further growth of the mycelium was obtained.

Several plates prepared in this manner were placed in a drying oven and heated slowly for 40 minutes. The temperature of the oven was thus gradually raised to 80° C. The plates were then withdrawn and allowed to stand in the laboratory at room temperatures. After 24 hours it was found that the spores thrown out upon the medium from the mature ascocarps before the cultures had been heated, had germinated and the mycelium was growing vigorously. This experiment was repeated with a number of plates, and unheated controls were maintained at room temperatures. In all the plates that had been heated the spores germinated, while none of the spores in the controls did so.

A series of experiments, some of which are tabulated below, were made to test more fully the effect of heat on germination and to determine the approximate minimum, optimum, and maximum temperatures for spore germination. Controls at room temperatures were maintained in ninety cases. It will be seen from the table that about 80 per cent of the spores heated to 60°–70° C. germinated. In no case were the spores in the controls even swollen. The method as finally worked out may be described as follows: Spores for inoculation were obtained by laying glass slides on corks over pieces of dung bearing ripe ascocarps. With the use of a Zeiss binocular, the spores were removed with a sterilized platinum needle and stabbed into the medium about 1.5 cm. from the edge. If more than one plate was to be inoculated it was found necessary to moisten the spores by blowing the breath on the slide, since they dry out rapidly and adhere to the slide so firmly that their removal is impossible without destroying them.

The species of Sordariaceae and Chaetomiaceae that usually grow in great abundance with species of *Ascobolus*, are for some reason not often present with *A. Winteri* on goose dung in this region. No spores of any other ascomycete were found on these slides. The precautions that are so necessary when pure cultures of other *Ascobolus* species are desired, are of little consequence when working with *A. Winteri*. The molds will be killed off, and the number of bacteria introduced with the spores will be much reduced by the heating process. Two ovens were used at different stages in my studies. One was a sheet-iron drying oven with an asbestos-lined shelf on which the cultures were placed. No oven of this type can be so arranged as to furnish the same degree of heat at all points on the shelf. By regulating a burner so that 20 minutes were required to raise the temperature of the oven to 75° C., as shown by a thermometer placed in one corner of the oven, and then removing the plates, good results were obtained. The plates were often left in the oven to cool, the door being opened, or the gas was turned off at 60° C. and the cultures allowed to cool in the closed oven. When this last method was employed, the spores themselves must have been maintained at temperatures between 50°–60° C. for at least 30 minutes. By substituting a burner that would raise the oven to 80° C. in five minutes, the per cent of germination was greatly reduced.

The second oven was porcelain-lined and was found to be less satisfactory for these experiments, because the temperature of the inclosed air would quickly rise to 100° C. or more while the agar medium would still be cool. To determine the temperature to which this oven must be raised in order to bring about germination, eight cultures were stacked one on the other in three different tiers. It was found that with the oven heated to 100° C. the spores in the lowest plates of the three tiers were the only ones that germinated. The other plates were then reheated to 65° C. in the sheet-iron oven and most of them gave positive results. It is not necessary to mention the great number of variations with which these experiments were performed. The size of the Petri dishes, depth of agar, and the position of the spores, are all factors which make it difficult to determine the exact temperature of the spore in each case where a solid medium is used. By quickly opening the oven

and stirring the agar with a thermometer it was found that when this oven had been heated to 75° C. during a period of 20 minutes the agar in the Petri dishes was at about 60° C. When water was substituted in place of the agar the plates in the front row on the asbestos shelf were only at about 55° C. while those on the rear row were at 60°–65° C. The evidence obtained, however, shows conclusively that the application of heat is an effective stimulus to spore germination.

The data for some thirty experiments, in which one hundred and ninety inoculations were made in as many plates, are summarized in TABLE II. The per cent of germination was determined by noting approximately how many spores of given groups failed to grow. 99 per cent means that ungerminated spores could not be found.

The plates in no. 20 and no. 21 were treated exactly alike but only 10 per cent of the spores in the first (no. 20) germinated, while practically all the spores in the second (no. 21) did so. The spores from no. 20 were obtained from dung collected in the field during very cold rainy weather. The apothecia and spores were very much paler than is normally the case. The plates of no. 21 were inoculated with spores from apothecia developed from dung that had been gathered the previous year and stored in the laboratory.

When an artificial culture has produced a number of ripe apothecia the spores may be seen lying all about on the surface and within the medium. These spores do not germinate even though the medium is well supplied with moisture. Three such cultures were heated to 60°, 65°, and 70° C. respectively. None of the spores in the medium germinated. It was not a case where the required nutrient was lacking, since many of the spores on the covers of these same dishes germinated in the film of water after being heated. No second crop of this species ever appears in damp chamber cultures. It may be, however, that certain toxic substances that inhibit germination are given off during the growth of the mycelium and apothecia. Spores from these same dishes were used to inoculate controls containing fresh media, and in these controls germination was abundant.

To determine the effect on germination when hard agar is used, a medium was made up with a much smaller percentage of

TABLE II

Results of experiments to show the effect of heat on the germination of the spores of *Ascobolus Winteri*

No.	Date	No. of plates	Medium, agar +	Minutes to heat oven	Final temp. of oven, C.	Germination observed	Per cent of germination
	(1910)						
1	Nov. 4, 4 P.M.	1	goose dung decoction	7	90°	Nov, 5, 8 P.M.	50
	Control	1	goose dung decoction	0	Room		0 (Nov. 8)
2	Nov. 4, 4 P.M.	1	goose dung decoction	7	90°	Nov, 5, 8 P.M.	20
	Control	1	goose dung decoction	0	Room		0 (Nov. 8)
3	Nov. 5, 9 P.M.	2	goose dung decoction	15	76°	Nov. 6, 3 P.M.	50
	Control	1	goose dung decoction	0	Room		0 (Nov. 8)
4	Nov. 12, 9 P.M.	1	goose dung decoction	15	76°		0 (Nov. 31)
5	Nov. 12, 10 P.M.	1	goose dung decoction	13	77°	Nov. 13, 9 A.M.	5
6	Nov. 12, 10 P.M.	1	goose dung decoction	13	77°	Nov. 13	99
7	Nov. 12, 10 P.M.	1	goose dung decoction	9	64°		0 (Nov. 25)
8	Nov. 12, 10:40 P.M.	1	goose dung decoction	17	82°		0 (Nov. 25)
9	Nov. 13, 12 M.	1	goose dung decoction	5	75°		0 (Nov. 15)
10	Nov. 14, 5 P.M.	1	goose dung decoction	15	77°		0 (Nov. 18)
11	Nov. 15, 5 P.M.	1	goose dung decoction	20	75°	Nov. 17, 1 P.M.	75
12	Nov. 15, 6 P.M.	1	Reheated No. 9	10	82°	Nov. 17	75
13	Nov. 18, 4 P.M.		Reheated No. 10	8	75°		0 (Nov. 30)
	(1911)						
14	May 28, 10 P.M.	18		15	70°	May 29, 8 A.M.	90
15	May 29, 11 P.M.	1	N/100 Na_2CO_3	15	70°	May 30, 9 A.M.	75
16	June 7, 11 P.M.	8	N/100 Na_2CO_3	25	65°	June 9, 8 A.M.	90
17	June 10, 5 P.M.	4	N/100 Na_2CO_3	31	60°	June 11, 8 A.M.	90
18	June 11, 5 P.M.	5	N/100 Na_2CO_3	35	60°	June 12	80
19	May 28, 11 A.M.	28	N/100 Na_2CO_3	30	65°	May 29, 9 A.M.	90
20	Sept. 25, 11 P.M.	30	goose dung decoction	15–20	60–75°	Sept. 26	10
21	Oct. 2, 10 A.M.	30	goose dung agar	15–20	60–75°	Oct. 3	99
22	Nov. 12, 4 P.M.	1	goose dung agar	11	72°		0 (Nov. 15)
23	Nov. 13, 11 A.M.	1	goose dung agar	15	75°	Nov. 14	99
24	Nov. 13, 12 M.	1	goose dung agar	30	90°		0
25	Nov. 15, 5 P.M.	1	goose dung agar	18	75°	Nov. 17, 8 A.M.	99
26	Nov. 19, 10 A.M.	1	goose dung agar	20	65°	Nov. 20	90
27	Dec. 17, 10 P.M.	1	goose dung agar	15	70°	Dec. 18	90
28	Dec. 20, 11 A.M.	20	horse dung decoction	17	70°	Dec. 21, 10 A.M.	90

water. In this case no surface film of moisture was visible on the agar nor on the cover of the dish. Twelve plates were prepared and the spores heated in the usual manner. No spores germinated. Small pieces (about 5 mm. square) of another agar medium containing a good supply of moisture were inserted in the plates of hard agar. Spores were then stabbed into the pieces of soft agar and heated. Practically all of the spores germinated. The mycelium grew out on the hard agar and produced a large number of apothecia in about ten days. This demonstrates the possibility of growth and reproduction under conditions not favorable for spore germination.

ASCOBOLUS CARBONARIUS Karst.

In my first experiments on the effects of heat on the germination of the spores of this species, small pieces of the apothecia were placed in Petri dishes on goose dung agar prepared as above described and placed in the sterilizing oven, which was then slowly heated to a temperature of 65° C., the time required being 30 minutes. They were then removed from the oven and left at room temperature. The following morning, in the cultures that had been heated, hundreds of spores had germinated; not only ripe spores, but half grown, hyaline spores had germinated, sending out fully as long and vigorous germ tubes as the others. In the unheated control plates there was no evidence of germination. A medium was then made up with an extract of heated soil as a nutrient. Twenty plates of this agar medium were poured and inoculated with spores taken from the glass slides. Ten of these plates were heated to 65°–75° C., the other ten were left at room temperatures. About 30 c.c. of the heated soil decoction was poured into each of twenty more Petri dishes and ascospores sowed in these. Ten of the dishes were heated to 65° C., and ten reserved unheated as controls. About 12 hours after, it was found that fully 90 per cent of the spores in both the liquid and the solid media that had been heated had germinated, while there was no germination in the unheated controls. Many small hyaline spores had also germinated. Comparison showed that these were half grown spores of *Ascobolus carbonarius* which had failed to reach maturity before being expelled from the asci. Further attempts to induce

the spores of this species to germinate at ordinary temperatures were unsuccessful.

To determine the temperature used in such experiments more exactly, two Petri dishes, each containing about 50 c.c. of a decoction of heated soil, were heated in the oven after having been well inoculated with spores. The temperature of the decoction was determined by stirring with a thermometer. The first reached 65° C. in 25 minutes, the second 60° C. in 19 minutes. So far as I was able to find, every spore in both dishes germinated. Another dish containing a like amount of the decoction without spores was heated in the oven for 30 minutes. At the end of this time the oven temperature was 80° C. The door was then opened and the temperature of the decoction proved to be 72° C. Spores were now put into this dish, the gas was turned off and the oven closed. After five minutes the temperature of the decoction was again determined as before. The temperature had dropped only two degrees. These spores were certainly exposed for five minutes to a temperature of 70°–72° C. Seven hours later all the spores had germinated.

In another experiment a beaker containing a decoction of heated soil was heated in a hot water bath to a temperature of 75°–76° C. Spores were then introduced and the temperature maintained at 75° C. for five minutes. Fully half the spores germinated after six hours and only about one per cent had failed to germinate at the end of 24 hours.

There is no doubt, however, that under certain conditions the spores of *Ascobolus carbonarius* may germinate without being exposed to such high temperatures. This is shown in the following experiment. A damp chamber containing a quantity of carbonaceous earth, upon which several ascocarps were growing, was exposed to direct sunlight for about two hours. Slides were placed above the ascocarps to catch the spores, and a few of these spores germinated in the film of water on the slide. A large number of the spores from these slides were wiped off into a heated soil decoction and kept at room temperatures. The spores that had already germinated continued to grow, and in addition about 5–10 per cent of the others germinated. It was found by further experiments that liquid exposed to the sunlight under these conditions

may reach a temperature of 50° C. in 30 minutes. This moderate rise in temperature has evidently a very stimulating effect on the spores, though the per cent of germination so achieved is not high.

In the following table (TABLE III) I have brought together a summary of the results of my experiments on the effect of heat on the germination of the spores of *Ascobolus carbonarius*.

The table shows that in the one hundred and twelve trials over 90 per cent of the spores germinated when heated in an oven for periods of from 15 to 40 minutes, the temperatures running up to but not exceeding 80° C. The highest per cent of germination was obtained by heating the oven up to from 70°–75° C. within the time limits specified. It is clear that the spores of this species very seldom germinate under cultural conditions unless heated to at least 50° C. for several minutes. Germination takes place equally well in such liquid media as tap water, decoctions of carbonaceous earth, decoctions of heated soil, and in dung decoctions; no difference in the percentage of germination is apparent when an agar medium made up with any of the decoctions just mentioned is employed, or when a peptone glucose agar or a malt agar is used. The percentage of germination is 90 per cent or over in all cases where the spores have been heated to 60°–75° C. for a few minutes.

To obtain a more definite idea of the minimum, optimum, and maximum temperatures for the germination of spores of *Ascobolus carbonarius* two series of experiments were made. (*a*) About twenty spores were placed in each of eleven test tubes half filled with a decoction of heated soil. A thermometer was used to stir the contents of the tubes while heating them separately in hot water baths. Each tube was heated for three minutes after its contents had been raised to the desired temperature, and was then cooled quickly by placing it in a cold water bath. After 24 hours the contents of the tubes were poured into watch glasses and the per cent of germinated spores ascertained by actual count. Observations made at the end of three days and again at the end of seven days showed that there was no further germination after the first 24 hours. TABLE IV shows the results of this set of experiments.

(*b*) In the second series the spores were allowed to remain on

TABLE III

The effect of heat on the germination of the spores of *Ascobolus carbonarius*

No.	Date	No. of plates	Medium	Minutes heated	Final temp. of oven, C.	Germination observed	Per cent of germination
1	June 10	3	Goose dung agar	30	65°	June 11	90+
	Control	3	Goose dung agar		Room		0
2	June 11	7	Heated soil agar	31	60°	June 12	99
	Control	7	Heated soil agar		Room		0
3	June 11	3	Goose dung agar	30	69°	June 12	90+
	Control	3	Goose dung agar		Room		0
4	June 12, 11 A.M.	1	Heated soil decoction	20	70°	June 12, 8 P.M.	99
	Control	1	Heated soil decoction		Room		0
*5	June 13	1	Heated soil decoction	20	66°	June 14	90
†6	June 15, 10 A.M.	1	Heated soil decoction	25	65°	June 15, 5 P.M.	99
7	June 15, 10 A.M.	1	Heated soil decoction	19	60°	June 15, 5 P.M.	99
8	June 21	2	Heated soil decoction		70°	June 21, 11 P.M.	90+
	Control	1	Heated soil decoction		Room		0
9	June 22	1	Heated soil decoction	30	80°	June 22, 10 P.M.	99
10	Sept. 4	6	Heated soil agar	15	70°	Sept. 5	99
11	Sept. 4	1	Heated soil agar		Room	Sept. 5	0.5
12	Sept. 27	12	Heated soil agar	40	100°		0
13	Sept. 28	6	Heated soil agar	35	90°	Sept. 29	50
14	Sept. 28	12	Heated soil agar	20	70°	Sept. 29	90+
15	Oct. 7	20	Heated soil agar	15	65°	Oct. 8	90
16	Oct. 7	20	Unheated carbonaceous earth decoction agar	20	70°	Oct. 8	99
17	Oct. 10	2	Peptone glucose agar	15	68°	Oct. 11	99
18	Oct. 10	2	Malt agar	15	68°	Oct. 11	99
19	Oct. 12	15	Decoction carbonaceous earth boiled 5 min. + agar	18	70°	Oct. 13	99

* The slide containing the spores used for no. 4 control was heated to 66° C. in the oven as indicated.

† In no. 6 and 7 the temperature of the decoction was determined by stirring with a thermometer.

The per cent of germination was determined approximately by observing the number of ungerminated spores in given groups.

the glass slides on which they had been caught from the apothecia, and each slide was immersed in a beaker of the decoction of heated soil, which had been raised to the required temperature. In this manner the time of exposure was in every case five minutes. At the end of this time the slide was removed and placed in a Petri dish containing a decoction of heated soil. A fresh decoction and clean beaker were used in each case. As these slides were covered with hundreds of spores, the percentage of germination was determined by counting the spores visible in the field of the microscope and an average taken of ten countings.

TABLE IV

Experiment to show the maximum, optimum, and minimum temperatures for the germination of the spores of *Ascobolus carbonarius* when heated 3 min.

Temperature, C.	No. of spores germinated	No. of spores not germinated	Per cent germinated
95°	0	16	0
93°	1	38	2+
90°	2	27	9+
83°	8	13	40−
80°	9	10	50−
75°	22	0	100
65°	7	0	100
60°	20	5	80
55°	11	10	50+
45°	1	17	5
35°	0	28	0
Room	0	19	0

TABLE V

Experiment to show the maximum, optimum, and minimum temperatures for the germination of spores of *Ascobolus carbonarius* when heated 5 min.

Temperature, C.	Per cent germinated
93°	0
90°	1
85°	30
80°	52
75°	90
70°	99
65°	99
60°	84
50°	60
45°	37
40°	3

Both series of experiments give the same general result. When the spores are heated for three minutes we find that only five per

cent of those raised to 45° C. germinated. All spores heated to 65°–75° C. germinated. Between 75° and 80° there was a rapid falling off and at 93° C. only one spore of the thirty-nine germinated. There was also a marked difference in the time required for germination to begin. In the cultures heated to 65°–90° C. the mycelium had already grown several millimeters when examined 24 hours later; the only spore germinated at 45° had just begun to send out the germ tubes at this time. When the time during which the spores were heated was lengthened to five minutes, the maximum temperature was lowered to 90° C. and the optimum was limited to 65°–70° C.

ASCOBOLUS VIRIDIS Curr. var. ?

Liquid decoctions were made from unheated soil gathered in the habitat of the fungus; other decoctions were made from this soil heated to 180° C. for one hour, and from alkaline soil from North Dakota both heated and unheated. Agar media were prepared with each of the above decoctions. At least fifty different plates were inoculated and subjected to various degrees of heat ranging from — 5° C. for 24–48 hours, to 25°–75° C. for much shorter periods. Germination was obtained only in drop cultures made in a decoction of heated soil from the locality where the plants grew. Of the thousands of spores in Petri dishes containing this same liquid, none germinated. The spores germinated in the drop cultures could not be induced to continue their growth on the agar media. FIG. 2 and 3 show the manner in which the epispore breaks up as the spore germinates.

ASCOBOLUS IMMERSUS Pers.

The method by which the spores of *A. immersus* were germinated is the same as was used with the preceding species. The results of my experiments are given in TABLE VI.

THECOTHEUS PELLETIERI (Crouan) Boud.

The methods by which this fungus may be obtained in cultures on the natural substratum have been well described by Overton (1906).

The large size of the spores and the fact that all of the thirty-two usually lie together on the slide arranged to catch them as they are ejected, make it possible to remove them without much danger of introducing the spores of other species. The agar medium was made

TABLE VI

The effect of heat on the germination of the spores of *Ascobolus immersus* Pers.

No.	Date	No. of plates	Medium, agar+	Time of heating oven	Final temp. of oven, C.	Germination observed	Per cent germinated
	(1910)						
1	Nov. 28	1	goose dung decoction	20 min.	75°	Nov. 30	25
2	Dec. 1	3	goose dung decoction	20 min.	75°	Dec. 3	+20
*3	Dec. 1	4	goose dung decoction	17 min.	75°	Dec. 3	100
4	Dec. 3	1	goose dung decoction	30 min.	80°	0	0
5	Dec. 19, 11 A.M.	1	goose dung decoction	30 min.	65°	Dec. 19, 9 P.M.	100
6	Dec. 19, 9:30 P.M.	1	goose dung decoction	30 min.	60°	Dec. 20, 9 A.M.	+
7	Dec. 21	1	heated soil decoction + Na_2CO_3 (N/50)	15 min.	65°	0	0
8	Dec. 21	1	heated soil decoction	15 min.	65°	Dec. 22	75
9	Dec. 21	1	dung decoction	20 min.	80°	Dec. 22	100
†10	Dec. 23	1	heated soil decoction	12 hrs.	−5°	0	0
11	Dec. 23	1	dung decoction	12 hrs.	−5°	0	0
	(1911)						
12	Apr. 19	3	goose dung decoction	20 min.	77°	Apr. 20	80
13	May 5	8	goose dung decoction	25 min.	75°	May 8	15
14	May 8	4	goose dung decoction	20 min.	75°	May 9	50

* Left the plates in the oven to cool. At the end of one hour the temperature of the oven was still 55° C.

† Two inoculated plates set outside the window during freezing weather for 12 hours were then maintained at room temperatures for 10 days without showing any signs of germination. As the table shows, in nearly every case where the plates had been heated for 15–20 minutes a large percentage of the spores germinated.

up with varying strengths of decoctions of dung and of heated soil, and the percentage of agar used was changed to get media of different degrees of hardness. The spores germinated fairly well after being heated 20 minutes, the final temperature of the oven being 70° C. They appear to germinate as well in the heated soil agar medium as in the dung decoction agar. A germ pore is present, as has been shown by Overton (loc. cit. *f. 15*). Germ tubes are usually put out at both ends of the spore at points only slightly to one side of the ends (FIG. 1). Four pure cultures of this species were allowed to grow for six weeks. The mycelium branched profusely in every direction and grew slowly and irregularly. Its appearance was such as to suggest that the nutrient medium was unsuitable for its normal development. After being subjected to daily examinations they became contaminated and were discarded.

The following table shows the results obtained from twenty-six plates inoculated and heated to 65°–75° C., the time ranging from 7 to 25 minutes. The low average per cent of germination (40 per cent) is accounted for by the fact that eight plates were overheated.

TABLE VII

Spore germination in *Thecotheus Pelletieri*

Date 1911	Number of plates	Agar medium + decoction	Time to heat oven	Final temp. of oven, C.	No. of cultures germinated	Positive results, per cent
Apr. 1	1	Goose d.		75°	1	100
*Apr. 4	8	Horse d.	20 min.	65–70°	6	75
Apr. 6	8	Goose d.	20 min.	70°	3	37
Apr. 13	1	Horse d.	7 min.	70°	0	0
†Apr. 19	8	Goose d.	25 min.	75°	1	12

ASCOBOLUS LEVEILLEI Boud.

Damp chamber cultures on horse dung produced such a large number of mature ascocarps that the spores could be caught directly by inverting an uncovered plate over the dung for an instant. In the first experiment three scattered clusters of spores were caught on the medium. The plate was heated to 68° C. in

* Plates were left to cool in the oven with the door closed. The temperature of the oven one hour after beginning the heating process was 55° C.

† The porcelain-lined oven was used. The eight plates were stacked one above the other. Spores in the bottom plate, the one most heated, germinated.

the oven. Two spores germinated (FIG. 27). The mycelium of this form is more delicate than that of the other species I have studied.

ASCOBOLUS LEVEILLEI var. AMERICANUS Cooke & Ellis. N. A. F. no. 1096.

I was unable to germinate the spores in cultures run parallel with those of *A. Winteri*. Five inoculated plates of horse dung decoction agar heated to 65°–76° C. likewise gave negative results.

ASCOBOLUS XYLOPHILUS Seaver.

This species was collected by F. J. Seaver in Colorado during the summer of 1911. It is one of the few species of *Ascobolus* that are said to grow on wood. An agar medium containing a decoction of heated soil was used. Eight plates were inoculated with small pieces of the dried ascocarps and heated to 55°–70° C. Only two germinated spores were found (FIG. 15). Small amounts of sodium carbonate were added to the medium, previously heated to only 55° C. and then reheated up to 70° C., with negative results. Another medium was made up with a decoction of decayed wood but this did not prove any more effective.

THELEBOLUS STERCOREUS Tode.

After two days, delicate growths of mycelium appeared in two cultures, described above, at several different points where the inoculation with the ascocarps of *A. xylophilus* had been made. No germinated spores of *Ascobolus* could be found at these points, but after eight days an abundant crop of the apothecia of *Thelebolus stercoreus* appeared on this mycelium. The apothecia were arranged in characteristic zones as figured by Ramlow (1906). The material in one plate was killed and imbedded for sectioning. The other plate continued to produce ascocarps for two months, remaining practically a pure culture of *Thelebolus*. Massee & Salmon (1902) and others have already pointed out that this species occasionally produces more than one ascus in an ascocarp. Such abnormalities as the production of the large spherical ascus directly from a cell of the ascogonium at a time before any of the enveloping hyphae had become visible, were also observed. Ramlow (loc. cit.) found that the spores germinated at ordinary temperatures. The circumstances under which the species appeared in my cultures

show that the spores will grow after being heated to 55°–60° C. for about 10 minutes.

SACCOBOLUS NEGLECTUS Boud.

The spores germinate readily after being heated to 60°–70° C. during a period of 15 minutes. Commonly one spore of the eight in the spore mass swells to a great size and sends out two or more germ tubes a little to one side of the ends (FIG. 6, 7). In their natural condition the spores are only slightly roughened, but after the swelling which precedes germination the spore wall is cracked in all directions and assumes a roughly warted appearance. In my experiments seldom more than four spores of the group of eight germinated. The mycelium develops in an agar medium containing a decoction of dung or heated soil and fruits well after about 10 days.

ASCOBOLUS FURFURACEUS Pers.

The ascospores which were used in the cultures of this species were secured from an isolated ascocarp of a typically furfuraceous form. A small quantity of sodium carbonate (1:500) was introduced into a plate of heated soil agar and the latter was again sterilized a few minutes at 90°–100° C. The plates were inoculated and the oven heated for 20 minutes, rising to a temperature of 65° C. The spores germinated very readily under these conditions (FIG. 29).

LASIOBOLUS EQUINUS (Müll.) Karst.

Mature ascocarps were crushed out in horse dung decoction agar and heated 15–17 minutes to 65°–68° C. A large number of spores in this plate germinated, but the culture soon became contaminated with bacteria and other fungi and developed no ascocarps.

ASCOBOLUS GLABER Pers.

Several forms of this species have been described. The one used in my work was not colored. The colorless *A. albidus*, which Massee & Salmon (1902) consider a variety of this species, is not, on the evidence of their description and figures of the germinated spores, the same that I have used. The irregular ridges of the epispore are shown in FIG. 26, *a*. I used a horse dung decoction agar in the one experiment tried. The plate was heated for 20

minutes in the oven, and the oven temperature reached 70° C. Many of the spores germinated within 24 hours. The germ tubes were well developed and the hyphae formed later were not distinguishable from the hyphae of *A. immersus*. Two views of a germinated spore are shown in FIG. 16, 26.

TABLE VIII

Species	Date	No. of plates	Medium, agar +	Time to heat oven, minutes	Final temp. of oven, C.	Germination observed
	(1910)					
Ascobolus Leveillei var. *americanus*	Nov. 6	1	horse dung decoction	10	76°	——
	(1911)					
Ascobolus Leveillei var. *americanus*	Oct. 8	4	horse dung decoction	15	65°	——
A. Leveillei	Apr. 7	1	horse dung decoction	17	68°	Apr. 8
A. glaber	May 5	1	horse dung decoction	20	70°	May 6
	(1910)					
Lasiobolus equinus	Nov. 28	2	horse dung decoction	20	80°	——
Lasiobolus equinus	Nov. 29	1	horse dung decoction	11	75°	Nov. 30
Ascophanus carneus	Nov. 15	1	horse dung decoction	15	75°	——
Ascophanus carneus	Nov. 7	1	horse dung decoction	10	70°	——
Ascophanus carneus	Dec. 20	1	horse dung decoction	18	69°	Dec. 21
	(1911)					
Ascophanus carneus	Sept. 29	3	goose dung decoction	15	74°	Sept. 30
Ascophanus sarcobius	Sept. 29	9	goose dung decoction	15	74°	Sept. 30
Ascobolus xylophilus	Dec. 21	3	N/100 Na_2CO_3+ wood decoction	20	60–70°	Dec. 23
Ascobolus xylophilus	Dec. 19	5	heated soil decoction	11	55°	——
A. furfuraceus	Dec. 21	3	heated soil decoction +N/50 Na_2CO_3	17	65°	Dec. 23
Thelebolus stercoreus	Dec. 21	2	heated soil decoction	20	60°	Dec. 23
Saccobolus neglectus	Dec. 24	1	heated soil decoction	16	65°	Dec. 25
Saccobolus neglectus	Dec. 24	1	horse dung decoction	16	65°	Dec. 25
Saccobolus neglectus	Dec. 25	1	horse dung decoction	20	71°	Dec. 26
Saccobolus neglectus	Dec. 30	1	goose dung decoction	16	74°	Dec. 31
Ascodesmis nigricans	Nov. 1	15	goose dung decoction	0	Room	Nov. 2
Ascodesmis nigricans	Nov. 2	2	goose dung decoction	7	50°	Nov. 3

TABLE VIII gives the data obtained in germination experiments with the several species just described. The percentage of germination was not recorded.

ASCODESMIS NIGRICANS Van Tieghem. (*Boudiera Claussenii* P. Henn.)

This species appeared in a damp chamber culture of *Gymnoascus ruber* and *Ascobolus viridulus* on dog dung, April 2, 1910. Several germinated spores were found on the slides used to catch the spores of the *Ascobolus*. The same fungus appeared on human excrement the following September, and a year later still another crop was found growing on goose dung. The spores germinate at room temperatures equally well in dung decoction agar and in a medium made up with decoction of heated soil. Some of the plates were heated to 45°–50° C. for 10 minutes without killing the spores. The mycelium grows rapidly and ascogonia and antheridia appear the second day. As many as ten concentric zones of fruits are formed in some plates. In this condition it resembles the culture of *Thelebolus stercoreus* described by Ramlow (1906), except that the zones of ascocarps are wider than the sterile spaces between adjacent zones. These two species are the only ones of the Ascobolaceae I have grown artificially that show such concentric zones.

The species of Discomycetes, *Detonia trachycarpa*, *Lachnea melaloma*, and *Plicaria violacea* were frequently found on carbonaceous earth along with *Ascobolus carbonarius*. Spores of these species did not germinate when heated to 65° C. in a heated soil decoction agar. *Ascobolus pusillus* (FIG. 8) is another species frequently found on old burned places. I tried two inoculations with spores of this species. The plates were heated to 65° C. None of the spores of *A. pusillus* germinated while practically all of the spores of *A. carbonarius* did.

As is well known, the spores of *Pilobolus* germinate in a dung decoction at normal room temperatures. I found that they would also germinate in a dung decoction agar when heated. This method killed off many of the bacteria and spores of other fungi, making it possible to get a fairly pure culture.

If one considers the evidence which has been given above of the conditions under which the spores of fourteen species have been

germinated, it will be seen that the artificial application of heat in certain cases is an effective substitute for whatever stimulus acts under natural conditions to induce germination.

We may now turn to the results obtained by the study of the development of the apothecia in certain of the species named above.

ASCOBOLUS CARBONARIUS Karsten, Fungi Fenniae exs. 463. 1866.

In 1866 Karsten issued as no. 463 of the Fungi Fenniae this species of *Ascobolus*, accompanied by a description which stated that the ascocarp was sessile, greenish, later brownish, and furfuraceous on the exterior. The spores were described as oblong-ellipsoid and purplish black. A formal description was published later (1870). This gave the spore measurements and added that they were reticulately sculptured and violaceous-fuscous in color. The habitat given for the species was on earth among pieces of charcoal. The identity of the species under this name seems to have been entirely lost sight of until the appearance of Seaver's paper on the Iowa Discomycetes (1910). Seaver concludes from a study of no. 463 Fungi Fenniae that the species commonly known as *A. atrofuscus* Phil. & Plow. is the same species. It has long been known that *A. carbonicola* Boud. (1877) and *A. viridis* Boud. (1869) are synonyms of *A. atrofuscus*. Durand (1902) has discussed the synonymy of *A. atrofuscus* and includes *Phaeopeziza Nuttallii* E. & E., N. A. F. no. 2908, among the synonyms. After having examined F. F. exs. no. 463, Phil. Elv. Brit. no. 47, E. & E., N. A. F. no. 2908, Fungi Galliae exs. no. 3935, and compared the spore markings and measurements with Boudier's figures and descriptions of *A. viridis* Boud. (1869, *pl. 5. f. 10*), I am convinced that our common species growing on burned places is identical with Karsten's *A. carbonarius*. Boudier has called our attention to a very characteristic abnormality which occurs in the formation of the outer layer of the spore wall. Such spores as he has figured (loc. cit., *pl. 5, iv, fig. 10*) can be found in all the exsiccati specimens referred to above, and in any collection brought in from this vicinity. FIG. 11 was drawn from a spore taken from Fungi Galliae no. 3935 (*A. atrofuscus*).

One reason for the confusion as to the species is found in Karsten's description of the spore markings. The surface is

at first covered with coarse warts and can not well be described as reticulated until the spore walls have dried out and cracked around the borders of the warts. In this condition the spores are reticulated, the reticulations, however, are formed by the cracking of the epispore. The only other species growing on burned places likely to be confused with *A. carbonarius* is *A. pusillus* Boud. (1877). *A. carbonarius* may be found from May until November growing where quantities of wood have been burned. Places burned in the autumn are favorable for the growth of this species during May and June.

As noted above, Boudier (1869) has given us a correct figure of a germinated spore of this species under the name *A. viridis*. Sometimes several germ tubes will arise from the middle of a spore instead of from near the end. The epispore is cracked in all directions, the smaller cracks running in between the warts and two or three larger cracks extending down to the endospore (FIG. 9, *a*).

The germinated spore becomes an integral part of the vegetative mycelium as was noted by De Bary (1884) for many Ascomycetes. FIG. 30 shows that the spore becomes a multinucleated cell, limited by the transverse septa formed beyond the points where the germ tubes emerged. Immature spores which germinated are shown in FIG. 31, *b*.

When the spores are germinated in a decoction of heated soil, and the mycelium is allowed to grow for two or three days in this liquid, there frequently appear at intervals along the course of the hyphae, swellings or sporelike bodies (FIG. 9, *b*, *d*). Such a body is first formed at the end of a hypha but immediately sends out another hypha from the opposite side so that it appears to have been formed as an intercalary swelling. They are probably merely vesicles such as are very commonly found in artificial cultures of all sorts of fungi.

About the third or fourth day one may look for the first appearance of a large number of spherical hyaline bodies arising at the extremities of straight narrow stalks, which are branches of the ordinary hyphae. They are borne singly, and as they are thin-walled and plainly function as spores they may be called conidia. These conidia are perfectly smooth and about 10 μ in

diameter. They may be asexual spores designed to spread the species more extensively over the burned area, or they may possibly be blown by the wind and then germinate under suitable conditions in regions far removed from the parent mycelium.

The conidiophore is slightly smaller at the end bearing the conidium (FIG. 9, *c*). One septum cuts off the conidium from its stalk and a second septum usually occurs at about the middle of the stalk. I am unable to state how these conidia are oriented when this species grows under normal conditions on carbonaceous earth. In artificial cultures on agar media and in liquid extract of heated soil they show little tendency to rise above the surface of the medium. They may be formed along the bottom of the Petri dish, within the medium itself, or may extend out above the surface.

A milk-white fluffy mass of mycelium always appears around the edge of the dish in cultures on agar. This mycelium is especially productive of the conidia. The conidia found on the surface of the medium are more or less pear-shaped and are bunched together on rather short threadlike stalks. Very rarely one finds a larger form bluntly pear-shaped and borne on an exceptionally long stalk (FIG. 45). It is not uncommon to see conidia with bladderlike outgrowths at the end or at one side (FIG. 10, 46, 48). In cultures several days old many of the conidia are entirely empty. This may have been due to a lack of sufficient nourishment or to some unknown pathological condition of the culture.

The method of the origin of the ascocarp in this species is unique among the Discomycetes so far studied. The process involves certain perplexing complications which occur at various stages, and we can come to a better understanding of what I believe to be the normal course of events by first describing what I consider a medial well-marked stage of development such as is shown in FIG. 33. The same letters are used throughout the description of earlier and later stages to designate particular parts of the initial apparatus in this species. FIG. 33 shows a spherical conidium (*a*), borne at the end of a rather stout stalk (*b*) which arises from a cell of a mycelial hypha. A septum cuts off the conidium from its stalk. The conidium has sent out a tube (*d*) nearly equal in

diameter to that of the conidium itself. There is a slight constriction at the point of emergence. The germ tube grows straight out for a distance of 50–90 μ and then forms a coil (*f*) of two to four turns. The cells of this coil all have the same diameter, each being three or four times as long as it is thick. The entire ascogonium at this stage consists of twenty to forty cells arranged in a loose irregular spiral tapering gradually toward the tip. The distal cell (*g*) of the stalk coil bends sharply and connects with the first cell (*h*) of the ascogonium by a large pore which is distinctly visible. The cell (*h*) is somewhat spherical and its diameter is much greater than that of the stalk cells. The distal cell (*j*) of the ascogonium grows out to form a cell (*k*) whose length is two or three times its diameter. This and the ten to twenty cells next beyond it form an organ (*l*) which it seems to me must be considered as a trichogyne. This trichogyne is more or less irregularly coiled and gradually tapers toward the end, which has coiled itself tightly about the upper part of a somewhat elongated conidium (*m*). The stalk (*n*) of this conidium is much longer and more slender than the stalk of the conidium (*a*). From the conidiophore (*b*) there arises a stout hypha, which sweeps out in an even curve, extending the entire length of the ascogonial coil. It sends out branches at intervals (*p*), which may anastomose with investing hyphae by H-shaped connections (*q*), and some branches come to lie in the region of the end cell (*j*) of the ascogonium. Other investing hyphae arise from the cells of the stalk coil. The stalk coil does not taper in either direction, and the sharp contrast in the shape, size, and contents between the cells *d* and *h* and between the cells *j* and *k* enables us to distinguish with certainty the limits of the three regions of the system, viz., the stalk, ascogonium, and trichogyne. The cells of these regions differ markedly in their behavior in fixing and staining. The cells of the stalk coil are dense and finely granular, are moderately darkened by osmic acid, and deeply and evenly stained by acetocarmin and safranin. The ascogonial cells have more coarsely granular and vacuolar contents. The cells of the trichogyne are nearly hyaline, are not blackened by osmic acid, and do not stain readily.

The connection between the tip of the trichogyne and the

conidium is very close. Whether fusion takes place can not be determined from the preparations as studied in glycerin jelly. There can be no question, however, as to the specific attraction between the trichogyne and the conidium, and I shall call it the antheridial conidium.

We may now note some variations from this type of development. FIG. 36 shows an archicarp in which the stalk is evenly coiled in a sort of snail-shell form. The conidium giving rise to the coil is concealed below but the stalk of the conidium is evidently at *b*. The ascogonium has made only one or two turns before growing far out in the medium. The outer portion has coiled upon itself and either come to an end at *j* or continued as a hyaline filament (*l*) which has come in contact with a mycelial hypha. This figure was drawn from the first fruit of this species that I had seen, and before the existence of the long transparent trichogyne was known. Since investing hyphae were being formed about the ascogonium, fertilization had evidently already taken place and the trichogyne, being dead, would scarcely have been visible without staining.

FIG. 34 shows an archicarp that has failed to develop further, probably owing to some injury received when an adjacent portion of the medium was removed. The first part of the stalk coil could not be found, and the remaining cells are entirely empty. The other regions, however, are very plainly marked, especially the connection between the stalk coil and the first cell of the ascogonium. The end of the trichogyne is plainly coiled around an antheridial conidium.

The young stages of a large number of ascocarps were examined and in every case in these cultures the ascocarp originates from a germinated conidium. This conidium can not be distinguished from the countless other conidia until it has germinated. The stalk is much stouter than in the case of the others. These female conidia may germinate in liquid extract of heated soil and form the stalk coil only. I have never been able to find the complete archicarp in a liquid medium. Occasionally one finds such cases of arrested development in cultures on agar media. FIG. 42 shows that after the stalk coil has been formed, it may take on a vegetative growth.

It is not always possible to trace the connection between the trichogyne and the antheridial conidium. In some cases the end of the trichogyne was quite plainly visible but the connection with the conidium could not be found. FIG. 35 represents a vigorously growing young ascocarp. The trichogyne (*l*) is very close to the long-stalked conidium (*m*). FIG. 40 shows a type in which the whole system is closely twisted into a tangled mass of hyphae, yet the trichogyne (*l*) is plainly seen coiled about a conidium (at *m*).

We have seen that the ascocarp originates as the result of the germination of a female conidium, the formation of a stalk coil, ascogonial coil, and a trichogyne. Whether fertilization regularly takes place by the fusion of the trichogyne with the second conidium is a question that can be determined only after further investigation.

In all the cultures containing ascocarps may be found a structure that at first sight might be taken for the stalk coil of the archicarp. It is, however, quite different in that it originates as a branch from a mycelial hypha, and after making a loose irregular coil of two or three turns sends off branches from various cells. It very frequently produces conidia either on short stalks arising from its cells (FIG. 48) or at the tip end of the coil (FIG. 47). Such coils have not been found in cultures not producing ascocarps, but are very common wherever fruits are being formed. They may be rudimentary archicarps.

One marked feature in connection with the formation of the vegetative portion of the ascocarp, is the presence of the hypha referred to above (FIG. 33, 35, 42) as arising from the stalk of the conidium that produces the archicarp. This hypha may, apparently in some cases, grow out of the conidium itself (FIG. 37, 44) or from the posterior cells of the stalk of the oogonium (FIG. 41). In either case it curves sharply over and extends along the coils of the ascogonium, coming to an end in the region of the trichogyne. This hypha and the stalk coil are still plainly visible in all young ascocarps, even after they have become comparatively well developed. No fusion was observed between this hypha and the trichogyne, although its structure and location might lead one to suspect that its function was not merely vegetative.

The growth of the apothecium proceeds slowly and several days elapse before ascogenous hyphae can be found. In the meantime a fringe of hyphae, the familiar secondary mycelium, grows out from the apothecium and penetrates the substratum in all directions. When this species is cultivated on carbonaceous earth in the laboratory, the base of the apothecium is clothed with a collar of light greenish yellow mycelium.

If the young apothecia are removed from the artificial medium and carefully crushed under the cover glass, the cells giving rise to the ascogenous hyphae may slip out and become exposed to view (FIG. 38). Staining with a drop of aceto-carmin serves to differentiate the paraphyses and the ascogenous system from the other tissues of the ascocarp. About three cells of the ascogonium nearest its stalk coil have become very much enlarged so that they are nearly spherical. The method of the formation of the ascogenous hyphae should be noted. Primary ascogenous hyphae first grow out from the second cell as stout spikelike growths, which are unbranched and consist of four or five cells each. When full-grown they are somewhat irregularly bent in the region of their tips. This is no doubt due to the obstructions they meet, otherwise they are perfectly straight (FIG. 39). Secondary ascogenous hyphae arise from the ends of each of these primary hyphae and branch a few times to produce the ordinary hook-shaped tips. The ascus arises from the penultimate cell while the ultimate cell may continue its growth and produce another ascus, or if this does not occur it bends sharply downward and becomes closely applied to the antepenultimate cell and possibly fuses with it. At the time when these primary ascogenous hyphae have attained about half their full growth, the next cell of the ascogonium, which is somewhat smaller than the one just described, begins to give rise to similar outgrowths, except that the basal cells may be somewhat swollen (FIG. 39, *r*). It is difficult to obtain both stages at the same time, consequently this cannot be said at present to be a constant feature. The swelling may have been due to the action of the aceto-carmin used in staining. The characteristic staining of the paraphyses and their close connection with these stalklike outgrowths from certain cells of the ascogonium may indicate a closer relationship than is now held to exist.

The ascocarps ripen slowly in the artificial media and reach maturity in 20–30 days after the spores have germinated. The largest ones grown artificially were 4–5 mm. in diameter. Spores from these have been used to obtain a second generation and do not appear to germinate without being heated. Ascocarps grown on the natural substratum in the laboratory require slightly less time to reach maturity and are usually larger than those grown artificially.

ASCOPHANUS CARNEUS Pers.

The cultures used in the studies of the origin of the apothecium of this species were obtained by transplanting small pieces of filter paper bearing the ascocarps to dung agar, where the mycelium grew rapidly and formed ascocarps sufficiently isolated to be transferred. In the first cultures large numbers of Zukal's sclerotia (1889), the gemmae of Ternetz (1900), and the chlamydospores of Cutting (1909) appeared. After several such transfers no such bodies were found. Nine pure cultures of a rough-spored variety, *A. sarcobius* Boud., from Bermuda, have not developed these chlamydospores. The ascospores of this variety are sufficiently large and well marked to enable one to remove them from the slide on which they are caught, with some degree of assurance that the spores of other species are not introduced into the medium at the same time. Three cultures containing the common smooth-spored form, all contain the "chlamydospores." Ternetz's careful investigation would indicate that the production of these bodies is characteristic of this species. Their non-appearance in certain cultures might be due to the nature of the medium used or to light and heat conditions. Or this may be good evidence that *A. sarcobius* is a very distinct species and not a variety of *A. carneus* as I at first assumed. Miss Ternetz's figures of the ascogonium agree with those I found in cultures obtained by transfer. The stalk (FIG. 17, *a*) is composed of about five to eight hyaline cells. The ascogonium is differentiated as a region of five or six larger, granular, and slightly colored cells. The ascogonium forms a loose coil of one or two turns (FIG. 17, *b*) and gives rise to a trichogyne which consists of five to seven narrow, non-granular cells. This organ is usually coiled tightly around over the body of the ascogonium but may sometimes be seen extending straight out

into the medium. When this occurs, one frequently finds that it gives rise to a slender branch at about the fourth cell. This side branch (FIG. 17, *d*) may in some cases curve over and come in contact with a hypha arising from the first cell of the stalk of the ascogonium, but I am unable to state at present whether this is a constant feature in the process of development. None of the cultures from the germinated spores has produced ascocarps. Ternetz has proved that *Ascophanus carneus* does not produce fruit when kept in darkness. My cultures were placed in a very dimly lighted room and this may account for their failure to produce ascocarps. Under the same conditions, however, *Ascobolus Winteri* fruited abundantly.

ASCOBOLUS WINTERI Rehm; Rabenhorst, Krypt.-Fl. 1^3: 1124. 1896.

The identification of this species was a matter of some difficulty. In some features it resembles *Ascobolus brunneus* Cooke, *A. stictoideus* Speg., *A. amoenus* Oud., *A. Leveillei* (Cur.) Boud., and *A. Leveillei* var. *americanus* Cooke & Ellis. The spores from apothecia grown in a vasculum are smooth, hyaline, and about 30 μ in length. They appear to be perfectly mature. The asci project from the ascocarp, and the spores are shot upward several centimeters. Such spores might be those of an *Ascophanus*. Spores in the asci from cultures in the light are pale amethyst colored. It is possible to so place this species with reference to the light that hyaline spores will be thrown off from apothecia on one edge of the substratum and well colored spores from the other. The spores are delicately warted; the warts being arranged in short broken lines give it a somewhat reticulated appearance, a characteristic of the spore of *A. brunneus* according to Rehm (1896). A few narrow cleftlike furrows in the epispore are visible (FIG. 12). These apparent clefts resemble those peculiar to the spores of *A. immersus* (FIG. 20). The young apothecia may appear to be either white, greenish, or cinereous, but this color is probably only that of the substratum showing through. The apothecia are rather transparent and colorless. Cf. *A. stictoideus* Speg. The fungus could not be identified with the descriptions of any known species.

In the Massee collection is a specimen accompanied with

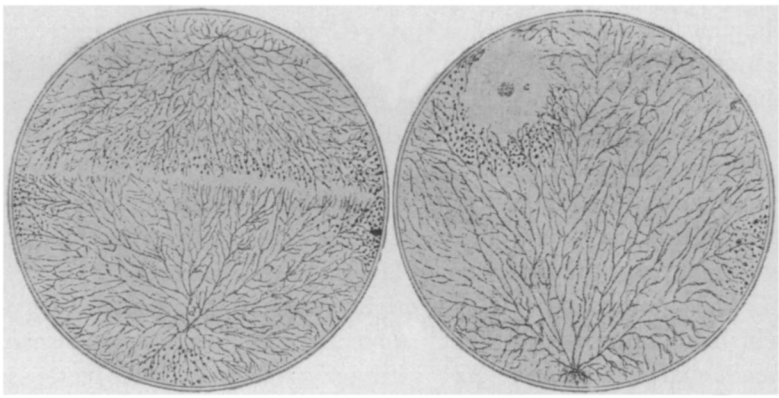
colored illustrations. The specimen is on goose dung and had been identified by Massee as *A. glaber*. His figure of the spores, and the spores themselves, agree with those in no. 211 Rehm Ascom. exs. (*A. Leveillei*) and with the spores of our species. Massee's specimen can not be *A. glaber*, because the purple spore markings of that species consist of ridges and not cracks. As is well known, Rehm afterwards decided (1896) that his *A. Leveillei* is a new species, which he calls *A. Winteri*. As noted, I was unable to identify this specimen from Rehm's description, but the agreement of my material with no. 211 Rehm Ascom. exs. is perfectly convincing.

Our species has been found on goose dung from several different localities in this vicinity but not on any other substratum. No other species of *Ascobolus* has been found with it, although abundant growths of *Ascophanus carneus* sometimes follow after a week.

An epidemic of cholera destroyed the geese at the Zoological Park in the summer of 1911. There were many other kinds of fowl around the same ponds after the epidemic but the fungus was not found on their dung. It was necessary to obtain material from other localities. It would appear that we have in this case one species, at least, that is closely confined to a particular kind of substratum.

The time that intervenes between spore germination and the formation of the initial organs of the apothecium, seems to depend mainly on two factors, viz., the nature of the medium and the point at which the inoculation has been made. If a weak decoction of dung or of heated soil is used in making up the agar medium a correspondingly longer time must elapse. If the inoculation is made at about 2 cm. from the edge of a Petri dish 10 cm. in diameter, the first apothecia will be found at a point between the germinated spores and the nearest edge of the dish. They have often appeared in this region in less than 48 hours after the spores germinated. On the other side, the mycelium will spread out fanlike and reach the edge in 4–6 days. Curiously enough, as a usual thing, no apothecia are formed until the growth of the mycelium is interrupted by the edge of the Petri dish; then they begin to appear in patches around the border. Later on, dense clusters may spring up at any point in the medium. Other regions

may remain entirely free from apothecia. When spores are sown at two points on opposite sides of the plate rather close to its edge, the hyphae from either region will not cross over into the territory of the other, but the apothecia are formed in an irregular line just back of the tips of the hyphae, parallel to a neutral zone between the two mycelia. (TEXT FIG. 1.)



1 2

FIG. 1. Shows the growth of the mycelium and formation of the apothecia of *Ascobolus Winteri* in a culture inoculated at two points on opposite sides of the plate.

FIG. 2. A large number of apothecia are formed in the region at *c*, where a colony of bacteria has developed.

The behavior of the vegetative hyphae with reference to bacterial colonies present in the cultures, also shows some interesting peculiarities. In some cases the mycelial hyphae would run through and over the bacterial colonies and mingle with them. In other cases the growth of the hyphae was checked at some distance from the bacterial colony, apparently owing to the emanation of toxic substances from it. A sterile zone one centimeter wide was sometimes left between the hyphae and the bacteria. Bordering this zone a dense aggregation of apothecia would form. (TEXT FIG. 2.)

No circular concentric zones of fruit bodies were formed in this species. Any factor tending to limit the vegetative growth of the mycelium serves to bring about the formation of apothecia.

The manner in which the mycelium made its way in a hard medium deserves notice. Ordinarily the mycelium grows along

the bottom, within the medium itself or on the surface. When a very hard agar is used, as described under spore germination, the mycelium from the germinated spores divides into two portions, one growing on the surface, the other along the very bottom. On focusing down through the medium the two planes of growth are seen to be very sharply defined. The older mycelium does not show the planes of separation as does the mycelium formed during the first few days. Another interesting feature in this connection is the vertical distribution of the ascocarps. In some cases all of the ascogonia were formed deep down in the medium and in direct contact with the bottom of the dish. Again they would form on the very surface of the medium or be distributed irregularly as regards depth. The amount of light and its direction, the temperature of the room, and the depth of the medium in the dish are apparently the determining factors in this respect.

The apothecium takes its origin in a well marked archicarp, which arises as a branch of a mycelial hypha and immediately bends sharply on itself. As growth proceeds its diameter increases to such an extent that it can be readily distinguished from ordinary vegetative branches (FIG. 50). A septum is formed a few microns from the parent hypha, and the distal part (FIG. 51) begins to curve at the tip and to form a spiral (FIG. 52) which ultimately consists of two or three complete turns (FIG. 53). This spiral may be formed on the same side of the hypha where it originated, or the branch may curve over or under the hypha and form the spiral on the opposite side. The coil continues to grow and thicken and soon becomes septate. Its tip becomes somewhat long and tapering, and on analogy with the carpogonia of the lichens may be regarded as a trichogyne. We can distinguish three regions in this initial organ (FIG. 54): the stalk (*a*) consisting of two or three cells; these, however, are not sharply distinguished from (*b*) one of the two or three larger central cells, which later becomes the ascogenous cells. The remaining outer portion of the spiral (*c*), which I shall call the trichogyne, is made up of three or four cells which gradually decrease in diameter and end rather abruptly (FIG. 53). At this time the cells of the coil are not inclosed by investing hyphae. A great many archicarps can be found in the stage of development shown in FIG. 53, and this may indicate that a short resting period

occurs at this time. I have not found that the archicarps are produced more abundantly during any particular hour of the day. From the first stalk cell a hypha (*d*) now arises which apparently grows very rapidly, keeping close to the coil (FIG. 54). Similar branches (*e*) then emerge from the second or even the third cell and these appear to be the first enveloping hyphae. At this stage the tip of the trichogyne may sometimes be seen to be entirely free but lying close to the next adjacent turn of the coil. Development from this stage on takes place so rapidly that it is difficult to follow the process. By crushing small portions of agar containing ascogonia under the cover glass and staining with acetocarmin or methylene blue, stages were found in which the tip of the trichogyne had actually applied itself to the branch from the lowest cell and perhaps fused with it. In several cases observed the point of contact is some distance back of the tip of this branch. It is quite possible that there is a fertilization at this stage and that the branch arising from one of the stalk cells is an antheridium. FIG. 55 shows the point of contact still farther from the tip than is ordinarily the case. When this cell was first examined no indication of a fusion could be made out, but when the cover glass was pressed down on the agar the spiral was partly untwisted and the apparently fused cells were exposed to view. FIG. 54 shows another similar fusion.

Many archicarps fail to produce apothecia. In these the spiral is more open and makes only one or two complete turns before a long tapering trichogyne is formed. The central cells are fewer in number, and the stout hyphae arising from the two or three stalk cells appear very early and extend straight out into the medium instead of up over the coil towards the trichogyne. The trichogyne tapers gradually into a slender tip (FIG. 57, *c*), or it may be set off sharply from the larger cells by a very sudden narrowing (FIG. 56). Such forms as these are very conspicuous and should be favorable for study. Although many attempts have been made to follow their development, no case was found in which the trichogyne came in contact with a hypha from the basal cells. As noted, none of these cases observed developed apothecia or continued their growth to any great extent. In one case the trichogyne was seen to be apparently fused with branches from neighboring

hyphae, and the branches from the basal cells were observed to fuse with similar vegetative hyphae. FIG. 57 shows such a fusion which resulted in no further development. Their failure to develop normally may possibly have been due to the treatment to which the plants were subjected in studying the cultures, which may have resulted in slight drying or even mechanical injury. Still in some cases archicarps were observed at about two-hour intervals continuously for two or three days, which showed but little further growth, while all about in the same cultures other coils were continuing their development and apothecia were being ripened. It seems quite possible that the failure of these archicarps to develop was due to failure of the trichogyne to effect a fusion with an antheridium, though the explanation of this failure is not clear.

ASCOBOLUS IMMERSUS Pers.

This species is widely distributed and very well known. The spores, which are about 60 × 30 μ, are entirely smooth with the exception of a few narrow clefts in the epispore (FIG. 20). FIG. 21 represents a spore germinating at four different points. The germ pores are distinctly visible at this stage. FIG. 22 and 23 show two germinated spores from the same group of eight. The hyphae shown in FIG. 23 were well fixed with a weak Flemming's fluid. The cytoplasm has a finely granular structure. The cytoplasm of the large hyphae (FIG. 22) is very coarsely reticulated and vacuolar. As the germ tube issues from the pore, it enlarges suddenly, giving rise to a hypha slightly thicker than the hyphae produced by some spores. These coarse hyphae can readily be distinguished in cultures on agar media. I have not learned that they differ functionally from the smaller and more common sort.

The mycelium grows vigorously in heated soil agar but produces very few fruits. The most abundant crops were obtained on a goose dung decoction to which sodium carbonate (1:500) had been added. It will be seen by referring to FIG. 24 that the archicarp is larger and contains more cells that the archicarp of *A. Winteri*. The structures shown in this figure are somewhat disproportionally swollen by the aceto-carmin stain, especially the

parent vegetative hyphae. The archicarp consists of about twenty cells, of which we may say four to six belong to the stalk, about eight to the ascogonial region, and the remaining cells to the trichogyne. A large number of archicarps were stained in toto with aceto-carmin and iron-hematoxylin. In a majority of these preparations there were three or four cells of the archicarp that took but little stain. All the other cells contained several nuclei while only an occasional nucleus could be found in the region of these hyaline cells (FIG. 25). I have seen no cultures that appear to be more vigorous that this one, and as there were many apothecia in all stages of development present, I can not believe this was a pathological condition. If such were the case it was pretty generally distributed in the culture.

In this species as in all others I have studied, the spiral nature of the coil is much altered as the ascocarp is developed. The investing hyphae push in between the turns and straighten out the coil so that sections of the apothecium show a wormlike body.

The young apothecia are covered with a secondary mycelium which spreads out in all directions, even directly upward to the surface of the medium when the apothecium is completely imbedded. The time required for the production of the mature fruit seems to be much longer in these artificial cultures than is the case with plants developing on the natural substratum.

ASCOBOLUS FURFURACEUS Pers.

The manner in which the spores germinate (FIG. 29) does not differ materially from that described for other species. Nine days after the germination, the cultures contained hundreds of archicarps and young ascocarps. The light greenish color was present at a very early stage. Molliard (1903) and Claussen (1905) found great numbers of oidia were produced on the mycelium. As has been noted above, the former was unable to obtain ascocarps without the introduction of bacteria. Claussen grew the mycelium from oidia for one hundred generations without obtaining fruits. I am very certain that no oidia were produced in these cultures, nor were there any chlamydospores such as Welsford (1907) has described. These asexual spores may possibly be

produced under different cultural conditions from those maintained in my experiments.*

The archicarp is very similar to that of *A. Winteri* and *A. immersus*. The three regions, stalk, ascogonium, and trichogyne, are not as distinctly differentiated as in the archicarp of *Ascophanus carneus*. The archicarp arises from the mycelium and forms a spiral coil of three or four complete turns. FIG. 28 is reconstructed from stained microtome sections. The few investing hyphae arising from the stalk cells and growing up near the trichogyne, are not included in the drawing. The stalk is quite well marked, consisting of about four short cells. The complete archicarp consists of about twenty cells.

GENERAL DISCUSSION

It is probably true that many species of the Ascobolaceae are distributed by animals that have eaten food upon which the spores have been ejected. It has been shown by Janczewski, Massee, and others, that animal digestion may be an effective stimulus to germination, or at least that the spores are not killed by this treatment. It is well known that the spores of *Thelebolus*, *Ascodesmis*, and species of *Rhyparobius* will germinate in dung decoctions or even in tap water without special treatment. Coprophilous fungi such as *Pilobolus*, *Gymnoascus*, and *Coprinus* germinate at ordinary temperatures; and it has not been proved that the species of *Ascobolus*, which have been thought to germinate only after being digested by animals, may not germinate readily at ordinary temperatures after a sufficient resting period. During a resting period of several weeks or months the possibly necessary chemical changes leading up to germination might be effected by natural agencies, such as the products of bacterial decomposition in the substratum, alternation of heat and cold, changes in condition of moisture, etc. My experiments show that occasionally the spores of a species will grow under conditions that are not, however, favorable for a general germination. These exceptional cases may account for the many contradictory statements that have been made

* Oidia have been found in several later cultures, presumably of *A. furfuraceus*, though they remained sterile and I have been unable to determine their identity with certainty.

regarding the germination of the spores of certain species of the Ascobolaceae, and any experiments on germination should take into account this apparent variability in the tendency of the spores to germinate under given conditions. None the less it is clear from my experiments that the spores of *Ascobolus* in general will not germinate in culture media at ordinary temperatures.

It has been recognized by many investigators that exposure to rather high temperatures favors the germination of spores as well as the germination of seeds. It is also well known that temperatures of 50°–60° C. do not kill the spores of certain species of fungi. I have been unable to find any account of germination effected at temperatures of 50°–60° C. when much lower temperatures would not have proved even more satisfactory. As the experiments here described have shown, the spores of certain species of *Ascobolus*, *Ascophanus*, *Thecotheus*, and *Lasiobolus* could be made to germinate abundantly by heating them to 50°–70° C. for a short time. The method of artificial culture that I have employed differs from others mainly in this fact, that I have subjected the spores to high temperatures for short periods as a means of inducing germination when longer exposures at lower temperatures were not effective. It may be that high temperatures bring about chemical changes analogous to the changes that might be produced at low temperatures during longer periods. It is a striking fact that half-grown spores of *Ascobolus carbonarius* germinated readily after being heated to 60°–70° C.

I have made a few experiments on non-coprophilous Discomycetes, which, however, were too limited to show whether the heating process was especially effective in connection with dung-growing fungi only. My experiments with *Ascobolus* suggest that rather unusual treatments may be effective in stimulating germination in the case of forms that have hitherto proved entirely resistant. It is especially interesting to note that heating not only favors germination but at the same time kills off other fungi which naturally grow along with the *Ascobolus* on the dung. Still certain species of the Sordariaceae germinate at ordinary temperatures in dung decoctions and many of these are not killed at these higher temperatures; in fact, some of them appear also to be stimulated by the process.

It will be of interest to determine whether there are temperature optima for spore germination in different groups of fungi, and also whether these optima correspond to those for enzyme action on fats and other reserve materials found in spores and seeds.

Melhus (1911), working with the spores of certain Oomycetes, has shown that the optimum temperature for the germination of spores of *Cystopus* is below that of the ordinary room and that the spores can be germinated abundantly and after being exposed to temperatures of 1°–5° C. for 24 hours. It is not easy to understand, why low temperatures should be effective in the case of the Oomycetes and high temperatures in the Ascobolaceae. The teleutospores of the rusts can be made to germinate much earlier if artificially subjected to low temperatures. Experiments which I have tried with *Ascobolus*, however, seem to indicate that freezing temperatures are not generally effective.

I have been able to germinate spores of several species of the Ascobolaceae in an extract of heated soil made slightly acid, and have noted a few spores germinating in a film of water on the cover of the Petri dish containing the apothecia. My experiments show that the acidity or alkalinity of a medium, within certain limits, or the amount of nutrient present, are entirely secondary factors and of minor importance in connection with spore germination, though, of course, these factors are highly important for the growth of the mycelium and the production of fruit bodies. As I have described above, the growth of the mycelium and the apothecia is apparently accompanied by the formation of certain toxins in the medium, which have an inhibitory effect on spore germination and on the growth of the mycelium from another region of the culture. This might seem to be opposed to Ferguson's observation (1902) that the spores of *Agaricus* can be made to germinate by placing small portions of the mycelium in the medium with the spores. The statements are, however, not necessarily antagonistic. The presence of an actively growing mycelium of the mushroom might hinder germination.

Janczewski (1871) has pointed out the weakness of a characterization of the Ascobolaceae on the basis of the projection of the asci above the surface of the hymenium. He finds that the asci of many species of the Pezizaceae likewise project above the

surface and that such a character is quantitative rather than qualitative. He suggests that it is only by a more thorough knowledge of the initial organs of the ascocarp that a satisfactory classification of the Ascomycetes can be obtained. Such a basis for classification would require a vast amount of investigation, since it is known that forms now widely separated have quite similar initial organs, and that in species now placed in the same genus these organs may be quite unlike.

The archicarp of *Ascobolus carbonarius*, with its long trichogyne conjugating with a conidium, is very suggestive of the conditions in the lichens. The trichogyne of the lichens, however, grows upwards through the tissues of the thallus until its tip becomes slightly protruded above the surface. The spermatia which are extruded from the spermogonia, are then in some way brought into contact with the tip of the trichogyne. It is probably true that many of the conidia arising from the mycelium of *Ascobolus carbonarius* are asexual spores, but it is quite as clear that some of them are functionally equivalent to the spermatia of the lichens. The presence of only a limited number of male cells, and these permanently attached to their stalks, would favor the development of a trichogyne with a tendency to grow outward in a very irregular fashion, thereby increasing their chances of reaching a male cell. In this similarity of the male cells to the ordinary vegetative reproductive cells we may have a step toward such a condition as is present in the rusts, where the sexual fusions occur between equal hyphal cells, and the spermatia have become functionless.

On the basis of these facts I am inclined to favor the view that the Ascomycetes have originated from the red algae through forms like the lichens, perhaps forms that have given rise to the lichens. Trichogynes and spermatia are found only in red algae and Ascomycetes, and the fungal element of the lichens represents closely the essential features of each group. This would not necessitate accepting the view that the apothecium is the most primitive type of the ascocarp. The differences between the cleistocarp and the apothecium are not very fundamental; the transition from the one to the other is easily conceived, and is indicated in the species of the lichens in which the fruit body is a pyrenocarp.

There are also forms in which the thallus is very poorly developed. Some species resemble quite closely species of *Humaria* and *Ascobolus* which grow on the ground among mosses and algae. An investigation of these forms may lead to the discovery of initial organs, which will even more convincingly establish the relationship between the Discomycetes and the discomycetous lichens.

I have shown that *Ascobolus carbonarius*, with its long trichogyne coming to wind about an antheridial conidium, is suggestive of a relationship between this species and the lichens. The development of the archicarp directly from a female conidium may be an adaptive feature correlated with the commonly occurring failure of the rudimentary coils (described p. 175) to develop ascocarps. No other ascomycete is known in which the archicarp originates directly from the germination of a conidium. I believe, however, that further investigation will show that this habit is not confined to this one species alone. When it is considered that only a few species of the Discomycetes have been cultivated artificially in such a way as to enable the investigator to follow the development of the apothecium directly from the mycelium, it is not surprising that our knowledge in this connection is very limited.

The multicellular trichogyne of *Lachnea stercorea* indicates a phylogenetic relationship between this species and *Ascobolus carbonarius*. Miss Fraser was unable to trace the origin of the antheridium with which this trichogyne fuses.

There is an undoubted tendency to the disappearance of the trichogyne in the Ascomycetes, though this does not necessarily mean a disappearance of sexual reproduction. We can trace the reduction of a trichogyne through well graded stages. Well developed septate trichogynes are also found in *Ascophanus carneus*, *A. ochraceus*, *Ascobolus immersus*, and *A. Winteri*. In the last two species the trichogyne is not always distinctly differentiated from the ascogonium. Spirally coiled archicarps tapering gradually toward the tip, are known in such forms as *Aspergillus*, *Sordaria*, *Hypocopra*, and *Saccobolus*.

In *Pyronema* the archicarp has become reduced to such an extent that septa are no longer found in the trichogyne. The antheridium has come to be developed sufficiently near the

oogonium to enable a one-celled trichogyne to bring the sexual nuclei together. The reduction of the trichogyne has gone on still further in *Ascodesmis*. *Humaria granulata* is one of the best known forms in which a trichogyne is no longer developed.

I am inclined to believe that Dangeard's species *Ascobolus mirabilis* is really *A. viridis* Boud., as he suggests it may be. I have given my reasons for considering this latter species as identical with *A. carbonarius* Karst. Dangeard found his species in one of his old cultures of *Pyronema* and assumed that it was introduced along with some carbonaceous earth upon which the *Pyronema* was growing. This habitat on carbonaceous earth is very characteristic of *A. carbonarius*. Dangeard found few apothecia in his cultures, a feature I have noted in my cultures of *A. carbonarius*. The structure of the archicarp, or as much of it as he saw, agrees in general with the central portion of that organ as I find it in *A. carbonarius*. The stalk cells are very similar, and the three or four enormous cells concerned in the production of the ascogenous hyphae are essentially like the same cells as I have described them. In a few cases he saw a few hyaline cells extending out beyond these larger cells, as do the empty cells of the distal end of the ascogonium in my forms. Dangeard noted how easily the ascogenous cells can be squeezed out of a young apothecium, and the appearance of the ascogenous hyphae growing out of the ascogenous cell was such as to attract his attention. He has also noted that septa are formed in these ascogenous hyphae as they grow out from the ascogenous cell, so that when they are mature they are straight, club-shaped structures consisting of three or four cells. These are plainly the organs that I have called primary ascogenous hyphae. His figure of a young apothecium, as seen from above, agrees also with my observations. What he has taken to be the remains of the stalk of the archicarp lying outside of the main fruit body, is in my opinion, however, the distal end of the archicarp from which the trichogyne grows. I find as above described, that this long winding end of the ascogonium becomes invested with hyphae quite independently of the portion that is to give rise to the main fruit body. It is only in the older stages that the whole system is uniformly inclosed to form an oval mass. The very transparent

trichogyne might easily escape his attention. He shows the archicarp arising directly from the mycelium as a branch of an ordinary hypha and found no cases in which it arises from a conidium. Both methods of origin occur beyond question. He makes no mention of asexual spores and describes the vegetative hyphae as being rather coarse and larger than the hyphae of *A. furfuraceus*. This is not always true for the hyphae of *A. carbonarius*. His description of the pores between the cells of the ascogonium does not agree with my observations on *A. carbonarius*, but he has not correctly described the pores as they exist in *A. furfuraceus*. If his species is not really *A. carbonarius* it is certainly interesting that there is another species so similar in many respects, growing on carbonaceous earth.

SUMMARY

1. The ascospores of many coprophilous species of the Ascobolaceae, which rarely germinate in artificial media under ordinary conditions, can readily be made to germinate by subjecting them to high temperatures, 50°–70° C., for five to ten minutes. In the case of *Ascobolus carbonarius*, which is terrestrial, many spores will still germinate when heated to 80° C. for five minutes. The heating process favors pure cultures, since the spores of many fungi are killed at these high temperatures.

2. Heating the spores appears to hasten the ripening processes; half-grown spores of *A. carbonarius* can be germinated in this manner.

3. Germination occurs about eight hours after the spores have been heated. The epispore becomes cracked in all directions, and two or more germ tubes are put out at short distances from the ends of the spore.

4. The acidity or alkalinity of a medium is not an important factor in determining germination. The number of apothecia produced may depend upon the reaction of the nutrient medium.

5. The mycelium of *A. carbonarius* produces a large number of conidia, some of which give rise directly to the archicarp. The archicarp consists of three distinct parts: the preliminary or stalk coil, the ascogonium, and the trichogyne. The tip of the trichogyne sometimes becomes coiled about an antheridial conidium. Archicarps may also arise from the mycelium.

6. The archicarps of *Ascophanus carneus*, *Ascobolus immersus*, *A. furfuraceus*, and *A. Winteri* arise directly from the mycelium; they are spirally coiled organs of which the peripheral cells represent a more or less strongly developed trichogyne. The trichogyne frequently becomes attached to a hypha growing out at the base of the archicarp.

7. The general character of the archicarps described and the presence of septate trichogynes, support the view that the lichens represent primitive forms of the Ascomycetes.

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Explanation of plates 10-15

The magnifications are given in connection with each figure. In some cases the surface markings of the spores are not shown in the figure.

PLATE 10

FIG. 1. *a*, germinated spore of *Thecotheus Pelletieri*, unstained. ×275. *b*, germinated spore of *Thecotheus Pelletieri*, showing germination from only one germ pore, stained in toto. ×425.

FIG. 2, 3. germinated spores of *Ascobolus viridis* var. ×450.

FIG. 4. *a*, immature spore of *A. viridis* var. ×800. *b*, mature spore of *A. viridis* var. ×800.

FIG. 5. Very large spore of *A. viridis* Curr. from *no. 196* Phil. Elv. Brit. ×800.

FIG. 6. Germinated spore of *Saccobolus neglectus*, showing the manner in which the epispore has been broken away during germination. ×450.

FIG. 7. Group of eight spores of *Saccobolus neglectus*, in which one spore has germinated and a second spore (*a*) has swollen preparatory to germination. ×450.

FIG. 8. Spores of *Ascobolus pusillus* Boud. ×800.

FIG. 9. *Ascobolus carbonarius: a*, germinated spore; *b*, swollen vesicle on the hypha; *c*, conidium; ×275; *d*, another vesicle stained with aceto-carmin; ×450.

FIG. 10. Conidium of *A. carbonarius*, showing swollen protuberance. ×250.

FIG. 11. Spore of *A. carbonarius*, showing the ends capped with a thickening of the epispore. ×800.

FIG. 12. Spores of *Ascobolus Winteri*. ×700.

FIG. 13. Germinated spores of *A. Winteri*. ×250.

FIG. 14. Mycelium and germinated spore of *A. Winteri*. ×725.

FIG. 15. Two spores of *Ascobolus xylophilus*, one spore germinated. ×450.

FIG. 16. Germinated spore of *Ascobolus glaber*, showing the manner in which the epispore becomes cracked due to swelling. ×400.

PLATE 11

FIG. 17. Ascogonia of *Ascophanus carneus: a*, stalk cells; *b*, ascogonium; *c*, trichogyne; *d*, hypha in contact with the trichogyne. ×250.

FIG. 18. Germinated spore of *A. carneus*. ×380.

FIG. 19. Germinated spores of *Ascophanus sarcobius*. ×380.

FIG. 20. Spore of *Ascobolus immersus*. ×400.

FIG. 21. Germinated spore of *A. immersus*. ×250.

FIG. 22, 23. Germinated spores and mycelium of *A. immersus*. ×650.

FIG. 24. Archicarp of *A. immersus*, stained in toto with aceto-carmin: *a*, stalk cells; *b*, ascogonium; *c*, trichogyne torn from the hypha (*d*). ×630.

FIG. 25. Archicarp of *A. immersus* stained with aceto-carmin, showing four apparently empty cells. ×630.

FIG. 26. Spores of *Ascobolus glaber: a* shows the characteristic markings of a mature spore; *b*, germinated spore. ×900.

FIG. 27. Germinated spores of *Ascobolus Leveillei*. ×650.

FIG. 28. Archicarp of *Ascobolus furfuraceus: a*, stalk cells; *b*, ascogonium; *c*, trichogyne. ×650.

FIG. 29. Spores of *A. furfuraceus: a*, *b*, ungerminated spores; *c*, *d*, germinated spores. ×360.

PLATE 12

Ascobolus carbonarius

The lettering for each figure is the same as given for FIG. 33.

FIG. 30. Section of a germinated spore of *A. carbonarius*. ×650.

FIG. 31. Germinated spore of *A. carbonarius: a*, optical section of a mature spore; *b*, immature spores which germinated. ×380.

FIG. 32. *a*, section of a conidium of *A. carbonarius; b*, sections of germinated female conidia. ×650.

FIG. 33. Mature archicarp: *a*, conidium giving rise to the stalk coil; *b*, conidiophore; *c*, hypha arising from conidiophore; *d*, first cell of stalk coil (*f*); *g*, last cell ot stalk coil giving rise to the ascogonium (*h*); *j*, last cell of ascogonium; *k*, first cell of trichogyne (*l*); *m*, male conidium or antheridium (?); *n*, stalk of antheridial conidium. ×400.

FIG. 34. Archicarp in a pathological condition. ×400.

FIG. 35. Archicarp in which the trichogyne (*l*) does not coil about the conidium (*m*), which is entirely empty. ×135.

FIG. 36. Archicarp in which the characteristic trichogyne was not seen; *l* may be the trichogyne. ×270.

FIG. 37. Archicarp in which the trichogyne lies beneath; *m*, a conidium near by. ×135.

FIG. 38. Cells of the ascogonium giving rise to primary ascogenous hyphae. ×400.

FIG. 39. Two adjacent cells of the ascogonium with primary ascogenous hyphae (*o*, *r*). ×400.

PLATE 13

Ascobolus carbonarius

Lettering as in FIG. 33.

FIG. 40. Mature archicarp showing the trichogyne (*l*) coiled twice about the conidium (*m*). ×300.

FIG. 41. Archicarp becoming invested with hyphae: a trichogyne is visible at *l*, the remaining portion being concealed beneath the ascogonium. ×300.

FIG. 42. The conidium (*a*) has germinated, giving rise to the stalk coil which has begun vegetative growth; *b*, the conidiophore; *c*, hypha arising from the conidiophore. ×300.

FIG. 43. *a*, germinated conidium with short thick stalk (*b*). ×300.

FIG. 44. Archicarp showing hyphae arising from the conidium (*a*) and from cells of the stalk coil (*d*); *j*, the last cell of the ascogonium giving rise to the trichogyne not shown in the figure. ×300.

PLATE 14

Ascobolus carbonarius

FIG. 45. Large pear-shaped conidium giving rise to slender hyphae. ×265.

FIG. 46. Conidium with a swollen tip. ×265.

FIG. 47. Coiled hyphae arising from the mycelium: *s*, the stalk of the coil; *t*, a conidium on the end of the coil. ×200.

FIG. 48. Two coils arising from the same mycelial hypha: *s*, the stalks of the coils; *t*, conidia arising from the coils; *u*, inflated conidia not connected with the coils. ×200.

FIG. 49. Coil arising from the mycelial hypha at *s*. ×200.

PLATE 15

Ascobolus Winteri

FIG. 50. Archicarp arising from the mycelium; the septum has not yet formed. ×675.

FIG. 51. One-celled stage of the archicarp. ×675.

FIG. 52. Archicarp composed of four cells, immature. ×675.

FIG. 53. Young archicarp showing hyphae (*e*) arising from the stalk cells. ×675.

FIG. 54. Mature archicarp: *a*, stalk cells; *b*, ascogonium; *c*, trichogyne; *d*, antheridium (?). ×765.

FIG. 55. Mature archicarp showing the trichogyne (*c*) and hypha (*d*) somewhat distorted by the pressure of the cover glass; connection distinctly visible. ×900.

FIG. 56, 57. Archicarps which have taken on a vegetative growth, the trichogyne (*c*) of FIG. 57 fusing with a mycelial hypha, and the hypha (*d*) from the stalk cell fusing with a branch from another hypha. ×675.

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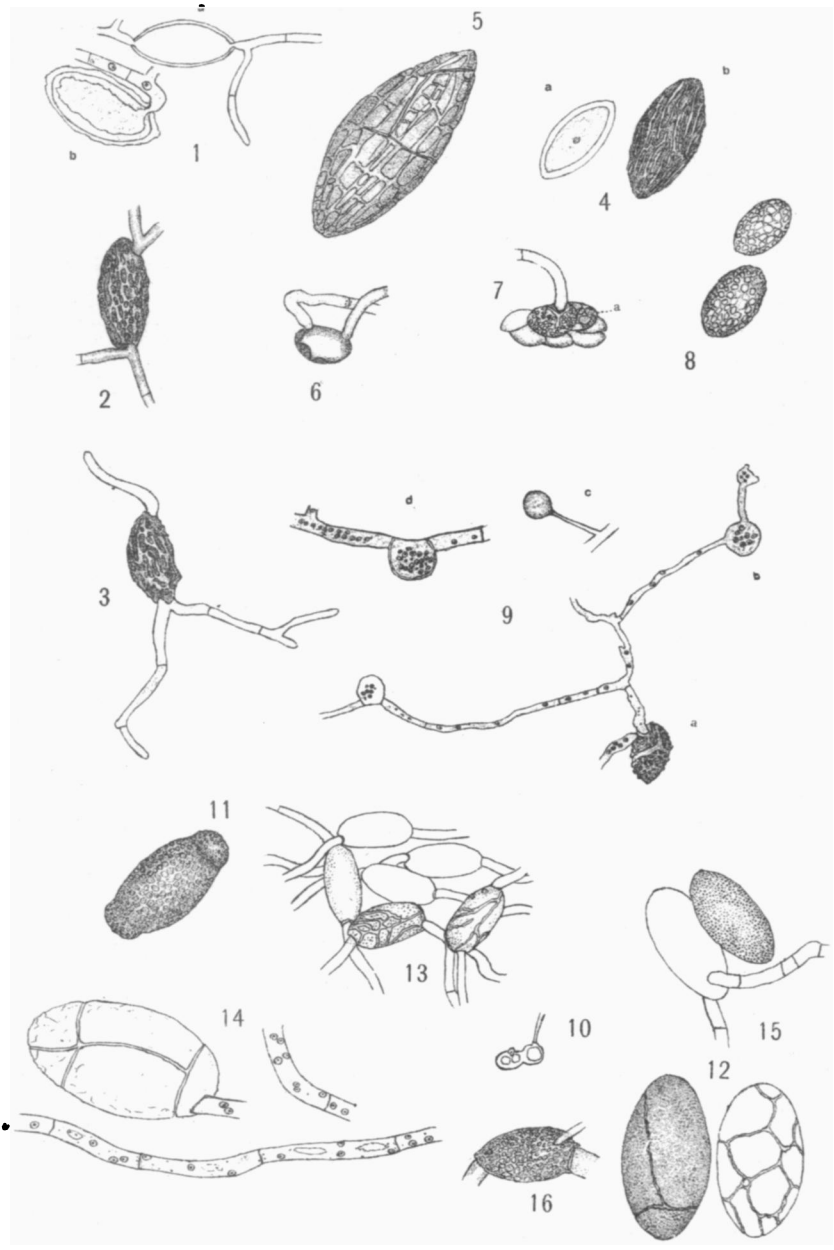

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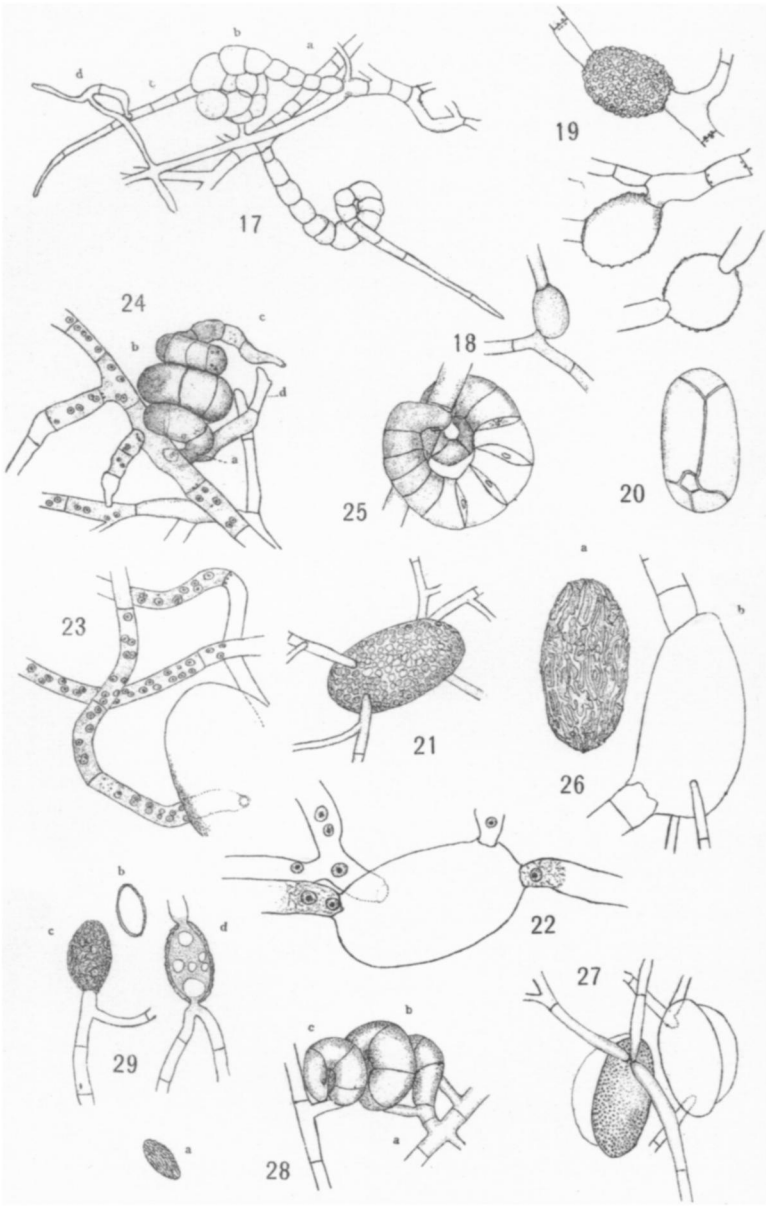

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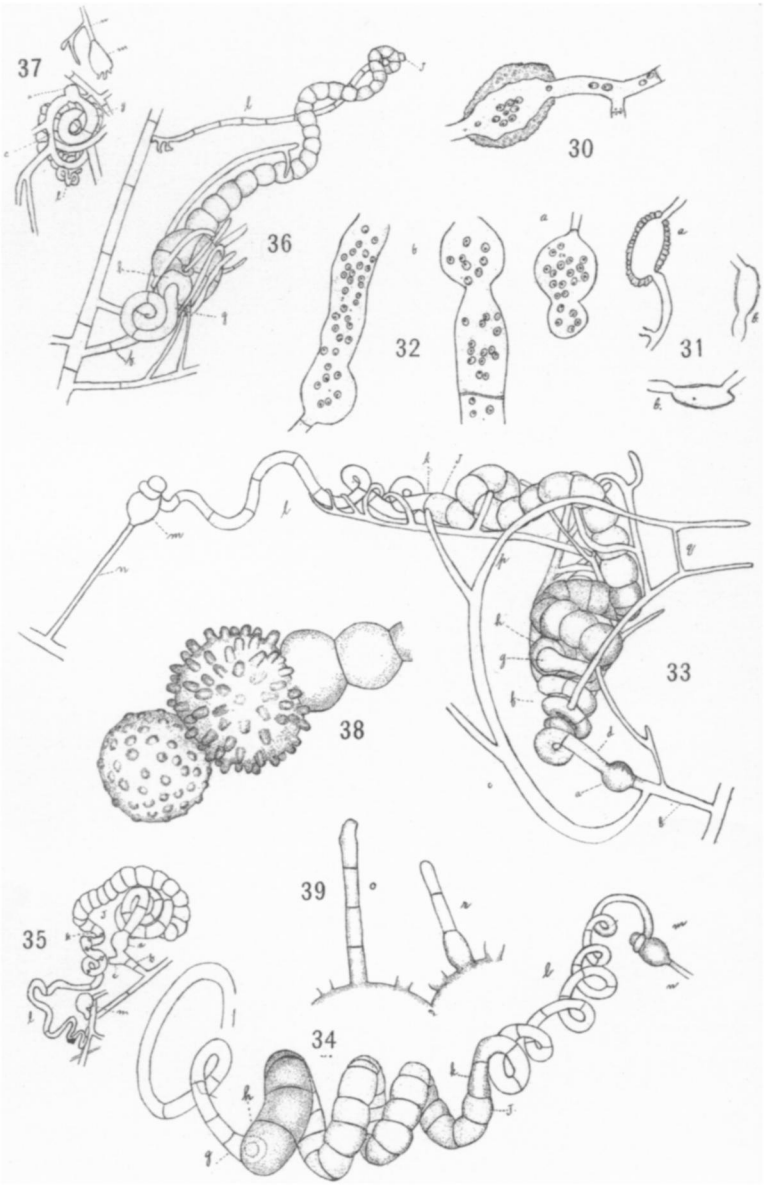

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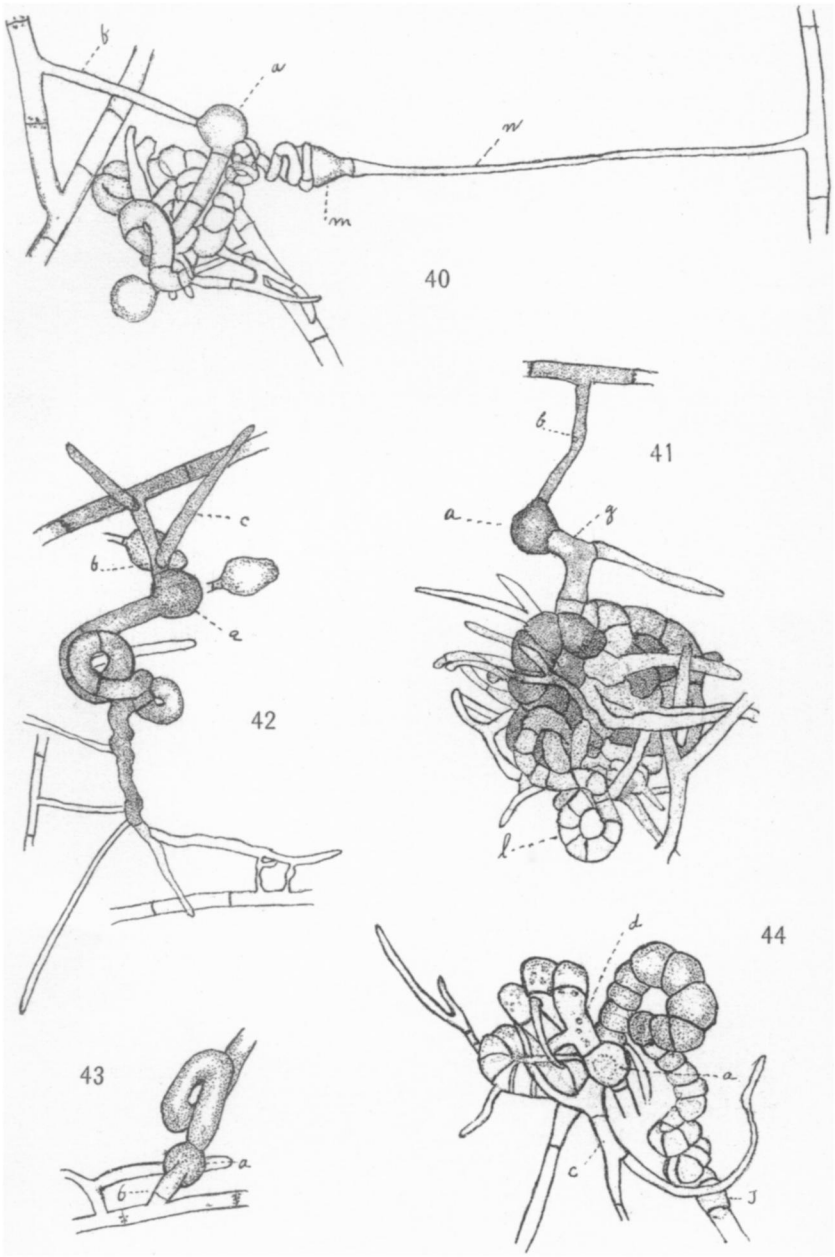

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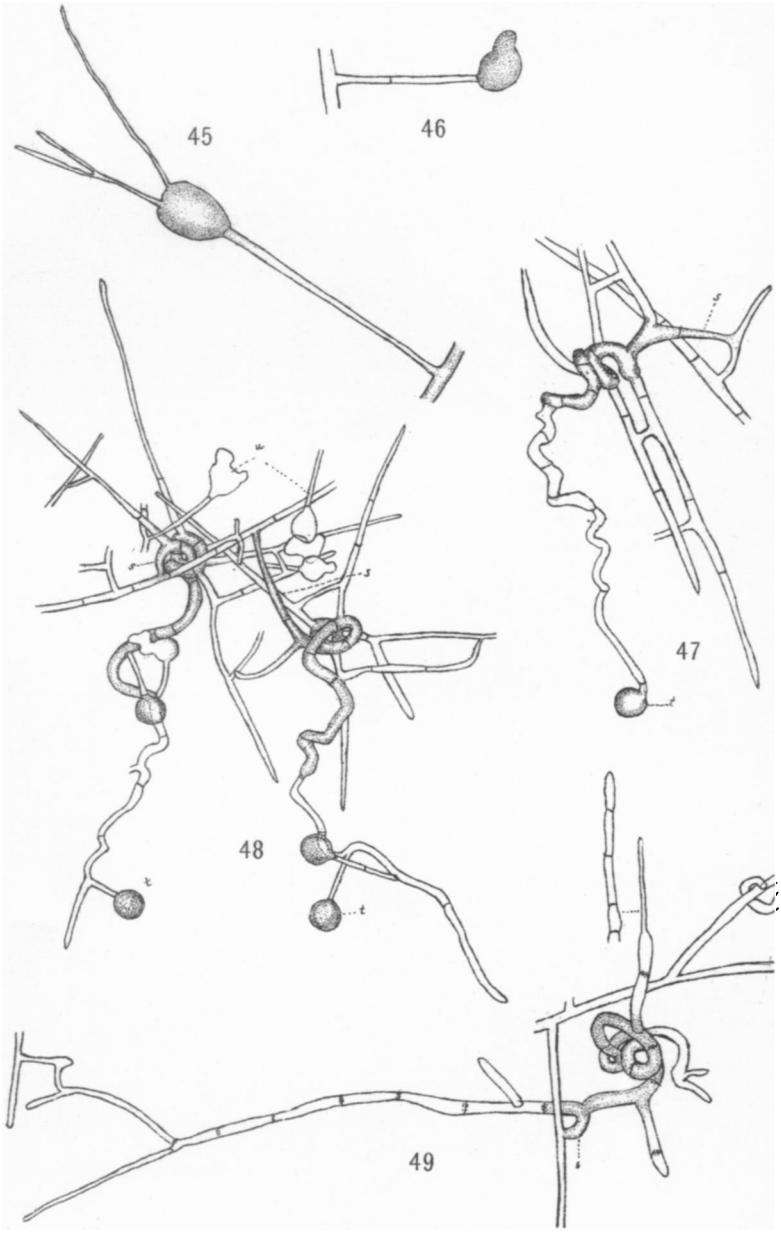

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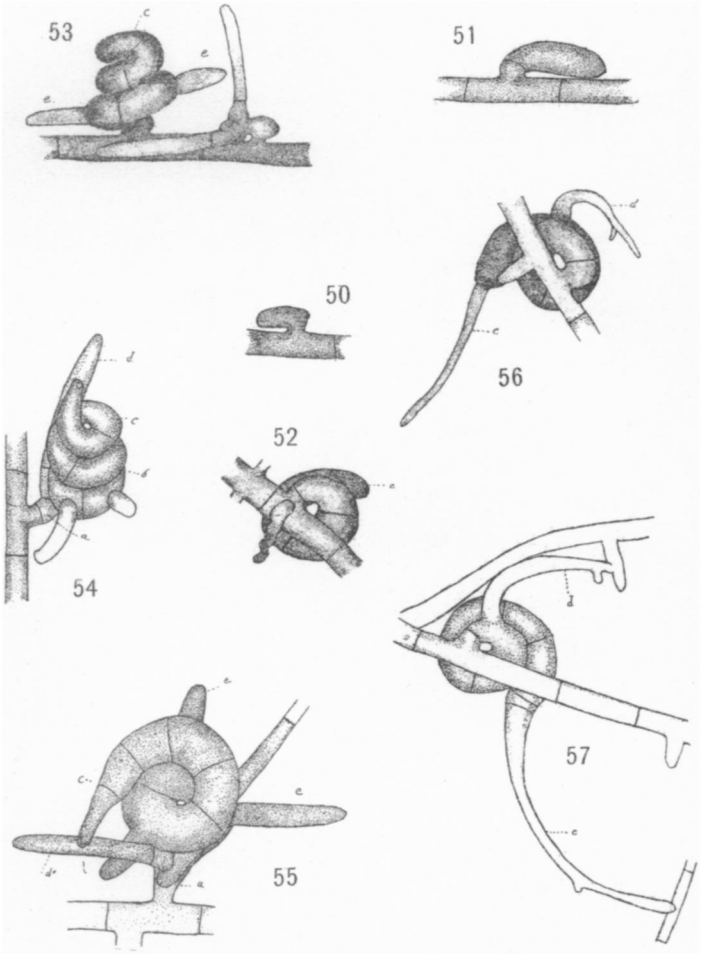

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